General aspects of the production and management systems and their relationship with *Corynebacterium pseudotuberculosis* infection and occurrence of mastitis of white Morada Nova sheep herds in semi-arid region

Aspectos gerais do sistema de produção e manejo e sua relação com a infecção por Corynebacterium pseudotuberculosis e ocorrência de mastite de rebanhos da raça Morada Nova branca em região semiárida

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ABSTRACT: This study aimed to characterize the production and management systems of white Morada Nova sheep herds and to present data on the occurrence of caseous lymphadenitis (CLA) and clinical mastitis (CM) in a population maintained in a semi-arid region. Blood samples were collected from adult females from herds of the Brazilian northeastern region. The first venous blood sample was used for a complete blood examination. The second blood sample was used to detect the presence of anti-*C. pseudotuberculosis* specific antibodies. The production and management systems of the herds were characterized using questionnaires, divided into three blocks of indicators: Facilities; Characterization of the production system; Health management. Body weight and hematological results were submitted to an analysis of variance, on which the fixed effect was clinical diagnosis, and the principal component analysis was performed to determine the main variables. For CLA, three herds stood out as having the highest rates of seropositivity (85.71, 79.41 and 60.45%). The same herds also had the highest occurrences of CM (9.30, 8.82 and 5.71%). The main variables that showed differences according the diagnosis of both diseases were blood granulocytes and lymphocytes. The study made it possible to observe the relationship between the best sanitary practices and the lowest occurrence rates of both CLA and CM. The diseases present considerable occurrence in sheep populations.

KEYWORDS: Caseous lymphadenitis; native breed; infectious diseases.

RESUMO: Este estudo teve como objetivo caracterizar os sistemas de produção e manejo de rebanhos de ovelhas Morada Nova da variedade branca e apresentar dados sobre a ocorrência de linfadenite caseosa (CLA) e mastite clínica (MC) em uma população mantida em região semiárida. Amostras de sangue foram coletadas de fêmeas adultas de rebanhos da região nordeste do Brasil. A primeira amostra de sangue venoso foi utilizada para um exame de sangue completo. A segunda amostra de sangue foi utilizada para detectar a presença de anticorpos anti-C. anticorpos específicos de *pseudotuberculosis*. Os sistemas de produção e manejo dos rebanhos foram caracterizados por meio de questionários, divididos em três blocos de indicadores: Instalações; Caracterização do sistema de produção; Gestão de saúde do rebanho. O peso corporal e os resultados hematológicos foram submetidos a uma análise de variância, na qual o efeito fixo foi o diagnóstico clínico, e a análise de componentes principais foi realizada para determinar as variáveis principais. Para o CLA, três rebanhos se destacaram com os maiores índices de soropositividade (85,71,79,41 e 60,45%). Os mesmos rebanhos também apresentaram as maiores ocorrências de MC (9,30, 8,82 e 5,71%). As principais variáveis que apresentaram diferenças de acordo com o diagnóstico de ambas as doenças foram granulócitos e linfócitos. O estudo possibilitou observar a relação entre as melhores práticas sanitárias e as menores taxas de ocorrência tanto de CLA quanto de MC. As doenças apresentam ocorrência considerável nas populações de ovinos.

PALAVRAS-CHAVE: Linfadenite caseosa, raça nativa, doença infecciosa.

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INTRODUCTION

Strategies to reduce morbidity and mortality rates in native herds are essential to decrease the number of breeds at extinction risk and consequently maintain the genetic diversity of these populations. Sheep farming has great economic potential in developing countries since it presents an excellent adaptation degree to extreme climatic conditions; an example of this situation is the Northeast region of Brazil, as it concentrates 65.5% of the national sheep population (IBGE, 2017). However, locally adapted native breeds, such as the Morada Nova sheep breed, are still little used as a consequence of their lower production rates when compared to exotic breeds. This specific breed represents an important genetic resource for the semi-arid region due to its high rusticity, precocity, high maternal ability and good performance in extensive breeding systems (FACÓ et al., 2008; NUNES et al., 2022).

Even in the face of these characteristics, the Morada Nova sheep breed had its population reduced over the years. For a long time, the animal selection criteria used by sheep farmers consisted of coat color, a situation that led to the discard of animals with black coat color; this practice is fully questionable, since the adaptive capacity of sheep of this breed is independent of coat color (FAÇANHA et al., 2021; LEITE et al., 2018). Consequently, the Morada Nova sheep breed of different varieties (red, white and black) is currently represented by a low number of animals and, according to the Brazilian Association of Sheep Breeders of Brazil (ARCO), only animals which have two types of coat colors (red and white) are registered.

The involvement of infectious diseases in herds generates leads to significant morbidity and mortality rates, and causes serious economic damage to the sheep meat and skin production chain, creating an obstacle to the activity. Some information on diseases linked to gastrointestinal parasites has already been reported in Morada Nova sheep (FERREIRA et al., 2017; 2018); indeed, there is an interest in studying other infectious diseases, such as caseous lymphadenitis (CLA) (ALVES et al., 2020) and clinical mastitis (CM) (PEREIRA et al., 2014), as they are the most commonly found diseases in sheep farms worldwide (SILVEIRA et al., 2021). CLA is a chronic disease caused by the bacterium Corynebacterium pseudotuberculosis, which is highly contagious and resistant to environmental conditions. The disease affects small ruminants as well as humans, and is considered an occupational zoonosis (BASTOS et al., 2012). On the other hand, CM has the bacterium Staphylococcus spp. as its main etiological agent. This disease occurs periodically in red Morada Nova sheep herds, but there are no scientific records in the white variety (FERREIRA et al., 2018). Selecting animals which are resistant to diseases that are frequent in production systems can to contribute to the survival of offspring and adult animals, and can decisively help maintaining the number of individuals in the herds.

Considering the situation mentioned above, it is necessary to carry out studies which seek to evaluate the health status of Morada Nova sheep herds white variety due to the low scientific production regarding the occurrence of diseases that affect the breed, raising data which encourage the elaboration of strategic actions aimed to the development of adequate sanitary management tools with the objective to minimize the morbidity and mortality rates of these animals. In this way, the objective of this study was to characterize the production and management systems of white Morada Nova sheep herds and to present data on the CLA and CM occurrence on a population of sheep raised in a semi-arid region.

MATERIAL AND METHODS

This experiment was approved by the Ethics Committee of the Universidade Federal Rural do Semi-Árido, Mossoró, Rio Grande do Norte state, Brazil (process identification: PED20001-2018).

The study was conducted on sheep production units in Rio Grande do Norte and Ceará states, Brazil, between 2020 to 2021. The climate in the region is characterized as tropical semi-arid (BSw' - Koppen climate classification), with the occurrence of two defined periods throughout the year: rainy (January to June) and dry (July to December). The annual average temperature and humidity are around 27-30°C and 40-80%, respectively, with a mean annual rainfall of 400 mm.

Eighty-two sheep were screened in this study, being that these sheep were located at six different production units in five different cities from in the Brazilian Northeast region (Table 1). Sixty-two sheep were screened for a second time one year after the first sample collection. The animals were 2 to 3 years old, non-lactating, non-pregnant and had a body weight average of 34.8 ± 6.4 kg, with body condition score around 2.5 (RUSSEL et al., 1984).

Information on the sheep production system and farmers were collected through a structured questionnaire based on the work of Loureiro (2012) and Silveira et al. (2022), with categorical and continuous variables applied *in loco*. The interviews were carried out with the farmers or technicians that were responsible for the management of the herd. The interview was

Table 1. Number of animals according to state and herd

Brazilian State	Herd	No. of animals
Rio Grande do Norte	Α	17
	В	34
	С	30
	D	13
Ceará	Е	35
	F	15
Total		144

divided into four blocks of indicators: (1) facilities; (2) characterization of the production system; (3) health management procedures; (4) and caseous lymphadenitis and clinical mastitis preventive measures. After completing the questionnaire, a technical visit to the farm was carried out to verify the reliability of the information collected.

Clinical examination was performed by palpation of the superficial lymph nodes and of the breast apparatus, by veterinarians specialized in small ruminant clinic. Two blood samples were then collected. The first venous blood sample was collected into vacuum tubes (BD, Franklin Lakes, New Jersey, United States) with ethylenediaminetetracetic (EDTA) disodium anticoagulant at 10% to evaluate hematological variables, including red blood cells (RBC, 106/mm3), mean corpuscle volume (MCV, fl), packed cell volume (PCV, %), lymphocyte count (LYM, 10³/mm³), monocytes (MON, 10³/mm³), granulocyte count (GRA, 10³/mm³), lymphocyte percentage (LY%, %), intermediate volume cell percentage (MI%, %), granulocyte percentage (GR%, %), red cell distribution width (RDWc, %), and white blood cells (WBC, 103 /mm3). The samples were analyzed in an automatized SDH-3 hematological analyzer (Labtest, Lagoa Santa, Brazil). The second sample was centrifuged at 2000 x g for 5 min and was used to detect the presence of specific anti-C. pseudotuberculosis antibodies through an ELISA using the recombinant protein PLD, as established by Barral et al. (2019), and characterized by a 91% sensitivity, 98.7% specificity and 96.5% accuracy.

The animals that presented lymphatic adenomegaly cases presenting a "mature lump" were submitted to fine needle puncture to collect caseous material. These samples were used to investigate the presence of *C. pseudotuberculosis* bacteria through the quadruplex PCR assay for species and biovar identification. The narG nitrate reductase gene was included in the assay along with the 16S, rpoB and pld genes to improve the diagnosis of multiplex PCR at the biovar level (ALMEIDA et al., 2017).

The CM diagnosis was performed by veterinarians specialized in small ruminant clinic by physical examination of the mammary glands, through inspection and palpation, according to criteria recommended by Grunert (1993). This diagnosis was based in the increase in volume and asymmetry between the glands, and in the characteristics of consistency of the breast parenchyma, such as the presence of nodules, edema, diffuse hardening and the presence or not of fibrosis.

The CLA and CM occurrences were calculated according to Wagner (1998), using the following formula:

[1]
$$Prevalence = \frac{Number of positive cases \times 100}{Number of the examined sample}$$

The data referring to the production and management characterization of the herds were analyzed according to the frequency of responses and expressed as a percentage. BW and hematological variables were submitted to analysis of variance (ANOVA) where the fixed effects were clinical diagnosis of CLA (1- Seropositive and no abscess; 2- Seropositive and with abscess; 3- Seronegative and no abscess; 4- Seronegative and with abscess) and CM (1- With fibrosis; 2- No fibrosis), and the ELISA results (Seropositive or Seronegative for C. pseudotuberculosis). The means of each variable were compared by the probability of difference using the Tukey's test at a probability level of 5%. Hematological data were standardized and multivariate analysis were performed. Principal component analysis (PCA) was performed to determine the main hematological variables. The relative importance of the variables was evaluated by the eigenvalues (variances) that were extracted by the orthogonal rotation (varimax method) and plotted on a two-dimensional graph. PCA reliability was tested using the Kaiser-Meyer-Olkin (KMO = 0.50) sampling adequacy measure and Bartlett's Sphericity Test (P < 0.001).

RESULTS

All farms have their own facilities for sheep, 50% of which originate from a cattle corral adapted for small ruminants, and 50% specific facilities were specific for sheep. Regarding the floor of the facilities, 66.7% have cement and soil floors, and 16.7% have mixed soil, cement and wood floors. The breeding system used on the farms is 66.7% extensive and 33.3% intensive breeding systems (Table 2). The animals grazed solely in Caatinga biome native pasture (50%), or native associated with cultivated pasture (50%). Regarding the concentrate supply, 33.3% provide corn bran, while 66.7% do not offer any type of concentrate feed. Mineralization is used in the animals' diet in all farms. The animals are identified through earrings (66.7%) or necklaces (16.7%), and 16.7% of the farms do not perform any type of animal identification. Only 33.3% collect birth data, and the offspring are naturally suckled on the ewes in all farms. For weaning age, 33.3% separate lambs from their ewes up to 3 months of age, 33.3% between 3 and 4 months of age, 16.7% between 4 and 5 months of age, and 16.7% above 5 months old. Regarding the separation of the herd into lots, 33.3% of the farms separate the animals by age group, and 33.3% separate pregnant females. Moreover, 50% of the farms separate the farrowing females, and 66.7% separate the animals by sex. The most frequent species that has direct contact with sheep herds are dogs (67.7%) and cattle (50%); however, it was cited the contact with cats (33.3%), horses (33.3%), goats (33.3%), wild animals (33.3%) and pigs (16.7%).

Most herds are vaccinating their animals (83.3%), and 50% vaccinate against rabies and clostridioses, and 33.3% only against clostridioses, while 16.7% do not vaccinate the herd with no vaccine at all (Table 3). All farms perform parasitological control with a frequency of one year, and only 66.7% of the breeders reported quarantining animals from other herds. The report regarding the fate of dead animals was necropsy (16.7%), buried (16.7%), cremated (33.3%)

or taken to places far from the farms' headquarters (33.3%). The vast majority of farms clean the facilities and only one farm does not carry out cleaning, followed by farms that carry out cleaning with a daily frequency (16.7%), three times a week (16.7%), monthly (16.7%), and once a year (33.3%). The managers reported the occurrence of CLA and CM in the last 12 months since the time of the visit. It was reported

that 83.3% of the farms presented cases of both diseases, and only one farm detected the occurrence of CLA only. Treatments were performed in all herds, but only 66.7% isolated the affected animals. It was reported that after removal, 50% of the farms bury and 50% incinerate the caseous CLA content. Most farms adopted wound cleaning (83.3%) and only the farm does not perform the procedure. The majority

Table 2. Characterization of the facilities and production systems of white Morada Nova sheep herds in Brazilian semi-arid region

Indicators/Variables		N	Frequency
Facilities			
Suitable for sheep	Yes	6	100%
Suitable type	Adapted cattle pen	3	50%
	specifically for sheep	3	50%
	Earthen floor	1	16.7%
Floor of the premises	Earthen floor and concrete	4	66.7%
	Concrete and wood	1	16.7%
Production system			
Tune of production system	Extensive	4	66.7%
Type of production system	Intensive	2	33.3%
Dacture	Native	3	50%
Pasture	Native and cultivated	3	50%
	Corn bran	1	16.7%
Supplementation	Other	1	16.7%
	No supplementation	4	66.7%
Mineralization	Yes	6	100%
	Earring	4	66.7%
Animal identification	Collar	1	16.7%
	No animal identification	1	16.7%
Birth data collection		2	33.3%
Suckling	Natural	6	100%
	Up to 3 months	2	33.3%
Manina	3-4 months	2	33.3%
Weaning	4-5 months	1	16.7%
	> 5 months	1	16.7%
Separation of the herds by batches	Yes	2	33.3%
Separation of pregnant females		2	33.3%
Separation of females from lamb		3	50%
Separation by sex		4	66.7%
	Dogs	4	66.7%
	Cats	2	33.3%
	Cattle	3	50%
Contact with other animals	Horses	2	33.3%
	Pigs	1	16.7%
	Goats	2	33.3%
	Wild animals	2	33.3%

(66.7%) reported that there were no reports of deaths caused by diseases; however, one farm reported one death caused by CM, and another farm reported one death caused by CLA.

The herd E (85.71%; 30/35), B (79.41%; 27/34) and C (76.66%; 23/30) stand out as presenting the highest seropositivity frequencies for CLA (Table 4). The total occurrence of CLA was 63.1%. The occurrence of CM was: D (15.38%; 2/13), B (8.82%; 3/34) and C (6.66%; 2/30). The total occurrence for CM was 6.25%.

Four animals with lymphadenomegaly were identified and had caseous material collected for the confirmation of the *C. pseudotuberculosis* presence, being one animal from the

herd B, two animals from the herd E, and one animal from the herd A. All samples were positive at the PCR specific for the presence of *C. pseudotuberculosis* at the lesion contents.

Table 5 shows the mean BW values and hematological parameters according to the diagnosis of CM. No statistical differences were found for the evaluated parameter between animals that presented or not clinical mastitis, However, when considering the sero-diagnosis of caseous lymphadenitis, it was observed that infected animals presented blood lymphocytes values statistically lower, and higher granulocytes counts than seronegative animals (Table 6).

According the PCA the two components, 51.18% of the total variation among the examined hematological parameters

Table 3. Characterization of the sanitary management and preventive measures for caseous lymphadenitis and mastitis of the white Morada Nova sheep herds in Brazilian semi-arid region

Indicators/Variables	N	Frequency	
(3) Sanitary management			'
	Necropsy	1	16.7%
5 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Landfill	1	16.7%
Dead animals' fate	Cremated	2	33.3%
	Discarded away from the farm	2	33.3%
	Rabies and Clostridioses	3	50%
Vaccine	Clostridioses	2	33.3%
	No vaccine	1	16.7%
Parasitology control		6	100%
Vaccination and parasitological control frequency	l year	6	100%
0	It has	4	66.7%
Quarantine	Does not have	2	33.3%
Cleaning of facilities	Perform	5	83.3%
	Does not perform	1	16.7%
	l per day	1	16.7%
Frequency of cleaning of facilities	3 per week	1	16.7%
rrequericy of cleaning of facilities	1 per month	1	16.7%
	per year or more	2	33.3%
Preventive measures - Caseous lymphadenitis and	l mastitis		
	Just Caseous lymphadenitis	1	16.7%
Occurrence	Caseous lymphadenitis and Clinical mastitis	5	83.3%
Animal treatment	Yes	6	100%
solation of affected animals	Yes	4	66.7%
Destination of compand and the	Incinerated	3	50%
Destination of removed nodules	Landfill	3	50%
-ibi	Yes	5	83.3%
njury hygiene	No	1	16.7%
	Caseous lymphadenitis	1	16.7%
Frequency of deaths	Clinical mastitis	1	16.7%
	No	4	66.7%

were explained (Figure 1). The variables that showed significant association with the first components contributed most to the total variation in the data and were in the following order of importance (based in component 1): %LY, %GR, GRA, MON, RDWc, MCV, LYM, WBC, PCV, %MI and RBC.

Table 4. Occurrence of caseous lymphadenitis and mastitis in the white Morada Nova herds in Brazilian semi-arid region

Geographic location	Samples (N)	Seropositive cases	Occurrence %
Caseous lymphadenitis			
А	17	2	11.76%
В	34	27	79.41%
С	30	23	76.66%
D	13	7	53.84%
Е	35	30	85.71%
F	15	6	40%
All	144	91	63.1%
Mastitis			
Α	17	0	0%
В	34	3	8.82%
С	30	2	6.66%
D	13	2	15.38%
Е	35	2	5.71%
F	15	0	0%
All	144	9	6.25%

DISCUSSION

The sheep farming in developing countries is characterized by traditional family farming (ARANDAS et al., 2020), that are inserted in extensive production systems, and is commonly managed in arid and semi-arid environments (SILVEIRA et al., 2021). The economic contribution of sheep production and animal productivity are naturally low in these production systems. The low efficiency of extensive systems in these regions is mainly due to climatic conditions and food shortages (DE KUMAR; BALAGANUR; NAQVI, 2020). The vast majority of farms evaluated in this study adopt this type of system. However, the information found herein is different from the findings by Guilherme et al. (2017), in which they found most production systems characterized by a semi-intensive regime. Their work was also conducted in the Brazilian semiarid region, where the authors justify this type of system due to prolonged drought periods, and with the objective to guarantee the maintenance of a viable activity.

Vaccination associated with the control of rabies and clostridioses were reported as the main prophylactic measures in the farms studied herein, in addition to the use of anthelmintics to control gastrointestinal parasites, as already reported by Ferreira et al. (2017) and Carvalho et al. (2020). Proper cleaning of facilities is also an important factor for controlling diseases in the herd (CARVALHO et al., 2020). The recommendation of cleaning the facilities is around once a day, at minimum (CRUZ et al., 2019), as the accumulation of waste, excreta and secretions can transmit a series of diseases to animals (ALMEIDA et al., 2010); however, only one farm in this study follows this recommendation.

Table 5. Body weight and hematological parameters of white Morada Nova sheep breed according to mastitis (CM) diagnosis

		Clinical diagnosis of the mastitis (No. of animals)	
		E (11)	F (131)
BW	kg	35.24 ± 8.55 °	34.19 ± 5.92 ª
RBC	106/mm³	9.05 ± 1.59 ª	9.44 ± 1.28 °
MCV	fl	41.18 ± 3.16 °	41.49 ± 3.54 °
PCV	%	35.59 ± 6.78 ª	36.95 ± 5.02 ³
LYM	10³/mm³	3.21 ± 1.35 °	3.29 ± 1.84 ^a
MON	10³/mm³	0.27 ± 0.15 °	0.27 ± 0.19 a
GRA	10³/mm³	2.60 ± 1.20 °	2.67 ± 1.81 °
LY%	%	52.90 ± 15.67 °	53.32 ± 16.59 ª
MI%	%	4.45 ± 2.03 °	4.52 ± 2.34 °
GR%	%	42.66 ± 15.72 °	42.16 ± 16.29 a
RDWc	%	27.57 ± 1.00 °	28.45 ± 1.54 °
WBC	10³/mm³	6.09 ± 1.66 ª	6.22 ± 2.63 °

ab Different letters on the same line indicate significant difference by the Tukey test with 5% probability

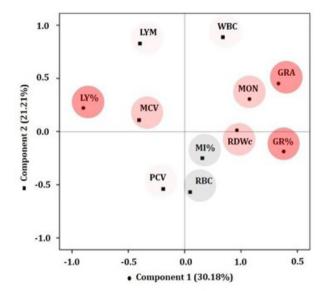
CM diagnosis: E- With fibrosis; F- No fibrosis; BW – Body weight; RBC – Red blood cells; MCV – Mean corpuscle (cell) volume; PCV – Packed cell volume; LYM – Lymphocyte count; MON – Monocytes; GRA – Granulocyte count; LY% – Lymphocyte percentage; MI% – Intermediate volume cell percentage; GR% – Granulocyte percentage; RDWc – Red cell distribution width; WBC – White blood cells

The results of this study show that the majority (66,7%) of farms perform quarantine. The importance of quarantine is to prevent the spread of important infectious disease agents, such as small ruminant lentiviruses and tuberculosis (GOUVEIA et al., 2015). The presence of dogs and cattle was frequently reported in white Morada Nova sheep herds. The presence of animals of other species, such as cats and pigs, is common on farms that do not develop a specialized livestock activity (ROCHA, 2016). Indeed, they can represent a reservoir of diseases for sheep and other animals (CARVALHO et al., 2020).

Most of the farms perform treatment of sick animals; however, the herds C and D claimed to not separate sick animals from the herd. Isolation consists of separating sick animals from healthy ones with the aim of reducing the risk of disease transmission, regardless of the infectious agent (SOUZA et al., 2018). A relationship was observed between the herds that did not undergo isolation through quarantine and the presence of the highest occurrence of the diseases studied herein.

The importance of investigations into the occurrence of CLA and CM is justified by the high morbidity and mortality rates of these diseases in sheep herds (GUILHERME et al., 2017). The economic damage generated to the production chain is factual, as the contaminated part of the carcasses are discarded, in addition to the disposal of animals confirmed with clinical signs as a way of controlling diseases in the herd (EMBRAPA CAPRINOS E OVINOS, 2021). Few serological studies were performed for caseous lymphadenitis in small ruminants using the iELISA diagnostic method, an assay that can detect asymptomatic animals (ALVES et al., 2020).

Furthermore, there are no studies which determine the sanitary condition of these animals for CM, which highlights the importance of such a study.



RBC – Red blood cells; MCV – Mean corpuscle (cell) volume; PCV – Packed cell volume; LYM – Lymphocyte count; MON – Monocytes; GRA – Granulocyte count; LY% – Lymphocyte percentage; MI% – Monocytes percentage; GR% – Granulocyte percentage; RDWc – Red cell distribution width; WBC – White blood cells

Figure 1. Two-dimensional plot of principal component analysis (PCA) referring to hematological parameters of white Morada Nova sheep breed.

Table 6. Body weight and hematological parameters of white Morada Nova sheep breed that presented seropositive or seronegative results for *C. pseudotuberculosis* infection

Maniablas	11	Mean \pm standard deviation	
Variables	Unit	Seropositive	Seronegative
BW	Kg	34.39 ± 6.46 ª	33.97 ± 5.55 °
RBC	106/mm³	9.42 ± 1.32 °	9.39 ± 1.28 °
MCV	fl	4 <u>1</u> .44 ± 3.69 °	41.45 ± 3.13 °
PCV	%	36.41 ± 4.93 °	37.60 ± 5.45 °
LYM	10³/mm³	3.01 ± 1.43 b	3.78 ± 2.24 °
MON	10³/mm³	0.28 ± 0.20 °	0.25 ± 0.14 °
GRA	10³/mm³	3.03 ± 1.95 °	2.00 ± 1.09 b
Y%	%	48.78 ± 15.12 b	61.49 ± 15.58 °
MI%	%	4.48 ± 2.23 °	4.60 ± 2.46 a
GR%	%	46.73 ± 14.75 b	33.92 ± 15.37 °
RDWc	%	28.46 ± 1.57 °	28.22 ± 1.44 °
WBC	10³/mm³	6.29 ± 2.50 °	6.03 ± 2.66 ª

ab Different letters on the same line indicate significant difference by the Tukey test with 5% probability

BW – Body weight; RBC – Red blood cells; MCV – Mean corpuscle (cell) volume; PCV – Packed cell volume; LYM – Lymphocyte count; MON – Monocytes; GRA – Granulocyte count; LY% – Lymphocyte percentage; MI% – Intermediate volume cell percentage; GR% – Granulocyte percentage; RDWc – Red cell distribution width; WBC – White blood cells.

The serological results indicate that there is a high frequency of seropositive animals, which is not surprising, as the Brazilian semi-arid region is endemic for the disease (SOUZA et al., 2011). A study on the epidemiology of CLA in an endemic region of Egypt showed similar results (SELIM et al., 2021). High CLA prevalence rates were reported in Brazilian regions (75.5% - GUIMARÁES et al., 2011; 77.9% - LOUREIRO, 2012; 83.4% - BARBOSA, 2016) and in other regions of the world with similar characteristics to those of our study (63.6% - ASLAN et al., 2016; 43.8% - ALGAMMAL, 2016). The use of barbed wire fences or cutting surfaces in troughs and trunks helps to justify the high CLA occurrence rates found in this study, in addition to the vegetation of the Caatinga biome being characterized by cacti which can cause injuries to the skin, which may be entry pathways of infectious agents (UNANIAN et al., 1985).

The recommended acceptable incidence of new CM cases should be less than 5% in small ruminants (OMALEKI et al., 2016). The incidence of CM was close to this range in this study. Silva et al. (2013) and Pereira et al. (2014) found positivity rates around 6% while studying CM etiologies in native breeds. However, it was expected that the herds studied herein would present lower occurrence, since they are kept in extensive systems and few herds perform weaning, and therefore the occurrence of CM would decrease as a result of the absence of residual milk in the mammary glands of the females (BRITO et al., 2007).

CLA in its visceral form is characterized by a severe decline in BW and respiratory manifestations, associated with chronic ruminal tympani (OREIBY, 2015). High frequencies of infected animals were observed in the present study, confirming the presence of the disease, not necessarily in its visceral form. Few abscesses were observed, suggesting that the disease may be present in its visceral form. Thus, it is believed that there is a high level of resilience of the animals, as no differences in BW were observed between seropositive and seronegative animals.

The high frequencies of seropositive animals suggest the low efficacy of the control and prophylaxis measures used in Brazilian herds of white Morada Nova sheep. The application of these measures varies according to the level of disease prevalence and the implementation of strict sanitary control (GUIMARÁES et al., 2011). The subclinical forms of the diseases are also factors which must be taken into account for the high frequencies found, since animals with the subclinical disease eliminate the infectious agent in the environment, acting as sources of infection in the herds (ALVES et al., 2020). Along with this, the chronic nature of the diseases allows that infected animal remain on the farm for a long time, a situation that is aggravated by the fact that they are small herds and there is not much turnover, and so young animals are at

greater risk of infection because they have more contact time with adults (ASLAN et al., 2016; ALVES et al., 2020).

Blood changes during infectious diseases can be discovered using hemogram, leukogram and serum biochemical indicators, providing an aspect of the animal's clinical health conditions (ODHAH et al., 2017). Most of the hematological parameters evaluated herein showed no difference according to the diagnosis, and no alteration was found in the case of CM. As for normality according to the reference values for sheep, a pattern very close to those suggested by Jain (1986) was observed. It was found differences regarding C. pseudotuberculosis infection, with a decrease in lymphocyte counts and an increase in blood granulocytes in seropositive animals, in a similar way that was reported by Mahmood et al. (2015) and Othman et al. (2014). This reversal of the relationship between GRA and LYM is a common response of ruminants to inflammatory processes (JAIN, 1986), and is a consequence of the extreme chemotaxis effect exerted by the C. pseudotuberculosis infection in different leukocyte populations, and even the production of innate immune system cytokines and acute phase mediators (BASTOS et al., 2012). Likewise, the importance of these variables and their inverse relationship are confirmed by the PCA, as well as the lower importance of variables such as PCV, %MI and RBC based in component 1. Despite being among the least important variables according to component 1, after observing component 2 we noticed an increase in the association with axis 2 of some of these variables, as WBC, LYM, RBC and PCV. According Ferreira et al. (2018) this is variables are important indicators for native ewes' health.

The hypothesis regarding CM is that the study cases could be considered chronic, arising from the cases that were evaluated in the clinical study, where there is no more inflammatory process, and therefore no leukocyte alterations (Latimer and Meyer, 1992). Thus, a more in-depth study with specific laboratory tests for CM investigating both clinical and subclinical cases is recommended for a greater depth of the study. However, there is a great need for this study, aiming to present the first results on the occurrence of the disease in white Morada Nova sheep populations.

CONCLUSIONS

This study made it possible to observe the differences between the farms that raise white Morada Nova sheep, especially in relation to the sanitary management condition, where those that carry out the best sanitary practices had the lowest occurrence rates of both CLA and CM. It is possible to affirm that the studied diseases are present in the different herds, even though they are small and isolated herds; there is great importance to obtain the occurrence of infectious diseases in the white Morada Nova sheep populations, since it can aid in planning appropriate measures to control them.

REFERENCES

ALGAMMAL, A. Molecular characterization and antibiotic susceptibility of *Corynebacterium pseudotuberculosis* isolated from sheep and goats suffering from caseous lymphadenitis. **Zagazig Veterinary Journal**, v. 44, n. 1, p. 1-8, 2016.

ALMEIDA, A. C. et al. Perfil sanitário dos rebanhos caprinos e ovinos no Norte de Minas Gerais. **Comunicata Scientiae**, v. 1, n. 2, p. 161-166, 2010.

ALMEIDA, S. et al. Quadruplex PCR assay for identification of *Corynebacterium pseudotuberculosis* differentiating biovar Ovis and Equi. **BMC Veterinary Research**, v. 13, n. 290, 2017.

ALVES, J. R. A. et al. Seroepidemiological study of Caseous lymphadenitis in sheep from the Northeast region of Brazil using an indirect ELISA. **Tropical Animal Health and Production**, v. 52, n. 4, p. 1945-1952, 2020.

ARANDAS, J. K. G. et al. Characterization of the sheep farming system in the Brazilian semiarid from the multivariate perspective. **American Journal of Animal and Veterinary Sciences**, v. 15, n. 3, p. 185-197, 2020.

ASLAN Ö. et al. Seroprevalence of caseous lymphadenitis in Kangal Akkaraman sheep. **Turkish Journal of Veterinary Animal Sciences**, v. 40, n. 6, p. 811-816, 2016.

BARBOSA, E. F. L. Soroprevalência e fatores de risco associados à infecção por *Corynebacterium pseudotuberculis* em pequenos ruminantes no estado de goiás. 2016. 53 f. **Dissertação (Mestrado)** - **Curso de Pós Graduação em Ciência Animal, Universidade Federal de Goiás, Escola de Veterinária e Zootecnia**, 2016.

BARRAL, T. D. et al. A panel of recombinant proteins for the serodiagnosis of caseous lymphadenitis in goats and sheep. **Microbial Biotechnology**, v. 12, n. 6, p. 1313-1323, 2019.

BASTOS, B. L. et al. *Corynebacterium pseudotuberculosis*: Immunological responses in animal models and zoonotic potential. **Journal of Clinical & Cellular Immunology**, v. 0, n. 0, p. 1-15, 2012.

BRITO, L. G. et al. Cartilha para o produtor de leite de Rondônia – Porto Velho, RO: **Embrapa Rondônia**, 2007. 40 p. – (Documentos / Embrapa Rondônia, ISSN 0103-9865; 116). Parte II: Aspectos técnicos.

CARVALHO, J. S. et al. Characterization of goat and sheep production in the state of Sergipe, Northeast of Brazil. **Acta Veterinaria Brasilica**, v. 14, n. 2, p. 121-131, 2020.

CRUZ, G. R. B. et al. Aspectos sanitários na produção de caprinos e ovinos de produtores familiares no semiárido paraibano. **Revista Conexão UEPG**, v. 15, n. 2, p. 129-134, 2019.

DE KUMAR, K. D.; BALAGANUR, K.; NAQVI, S. M. K. Effect of environmental factors on estrus synchronization and artificial insemination success in farmers flock in sheep under semi-arid tropical region. **Reproduction in Domestic Animals**. V. 55, n. 7, p. 777-784, 2020.

Embrapa caprinos e ovinos. **Centro de Inteligência e Mercado de Caprinos e Ovinos. Linfadenite Caseosa (LC)**. Disponível em: https://www.embrapa.br/cim-inteligencia-e-mercado-de-caprinos-e-ovinos/zoossanitario-linfadenite>. Acesso em: (28.dez.2021)

FAÇANHA, D. A. E. et al. Thermoregulatory responses, acid-base and electrolytic balance of indigenous ewes of different coat colors in a equatorial semi-arid region. **Animal Production Science**, v.62, n.2, p. 121-130, 2021.

FACÓ, O., et al. Raça Morada Nova: Origem, Características e Perspectivas. Sobral – CE: **Embrapa Caprinos e Ovinos**, 2008.

FERREIRA, J. B. et al. Performance, endoparasitary control and blood values of ewes locally adapted in semiarid region. **Comparative Immunology, Microbiology & Infectious Diseases**, v. 52, p. 23-29, 2017.

FERREIRA, J. B. et al. A multivariate approach to the diagnosis of gastrointestinal infection in ewes. **Veterinary Parasitology**, v. 252, n. 15, p. 95-97, 2018.

GOUVEIA, A. M. G. et al. Zoo-sanitary aspects of goat husbandry in Southeastern Brazil. **Semina: Ciências Agrárias**, v. 36, n. 1, p. 277-284, 2015.

GUILHERME, R. F. et al. Characterization and typology of sheep and goat production systems in the State of Paraíba, a semi-arid region of northeastern Brazil. **Semina: Ciências Agrárias**, v. 38, n. 4, p. 2163-2178, 2017.

GUIMARÃES, A. S. et al. Caseous lymphadenitis: epidemiology, diagnosis, and control. **IIOAB Journal**, v. 2, n. 2, p. 33-43, 2011.

GRUNERT, E. Sistema Genital Feminino, p.269-314. IN: DIRKSEN G.; GRÜNDER H. D.; STÖBER M. (Eds), Rosenberger: Exame Clínico dos Bovinos. **Guanabara Koogan**, Rio de Janeiro. 1993.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE). **Censo Agro**, 2017. Disponível em: https://censos.ibge.gov.br/agro/2017>.

JAIN, N.C. **Schalm's Veterinary Haematology**. 4th Edition, Lea and Febiger, Philadelphia, PA, 1221, 1986.

LATIMER, K. S.; MEYER, D. J. Os leucócitos na Saúde e na Moléstia. In.: **ETTINGER**, S. J. Tratado de Medicina Interna Veterinária. 3 ed. São Paulo: Manole, 4:2616-2664, 1992.

LEITE, J. H. G. M. et al. Locally adapted Brazilian ewes with different coat colors maintain homeothermy during the year in an equatorial semiarid environment. **International Journal of Biometeorology**, v. 62, n. 9, p. 1635-1644, 2018.

LOUREIRO, D. Soroepidemiologia da linfadenite caseosa, da doença da língua azul e da doença de maedi-visna em ovinos de raça definida do estado da Bahia e correlações com aspectos zootécnicos. 2012. 98 f. Dissertação (Mestrado) – Curso de Pósgraduação em Ciência Animal nos Trópicos, Universidade Federal da Bahia, 2012.

MAHMOOD, Z. et al. Assessment of blood changes postchallenge with Corynebacterium pseudotuberculosis and its exotoxin (phospholipase D): A comprehensive study in goat. **Veterinary World**, v. 8, n. 9, p. 1105-1117, 2015.

NUNES, S. F. et al. Fine genetic structure of Brazilian White Morada Nova hair sheep breed from semi-arid region. **Small Ruminant Research**, v. 221, p. 106694, 2022. ODHAH, M. N. et al. Hemogram responses in goats toward challenged with Corynebacterium pseudotuberculosis and its immunogen mycolic acids. **Veterinary World**, v. 10, n. 6, p. 655-661, 2017.

OMALEKI, L. et al. Molecular epidemiology of an outbreak of clinical mastitis in sheep caused by Mannheimia haemolytica. **Veterinary Microbiology**, v. 191, n. 15, p. 82-87, 2016.

OREIBY, A. F. Diagnosis of caseous lymphadenitis in sheep and goat. **Small Ruminant Research**, v. 123, n. 1, p. 160-166, 2015.

OTHMAN, A. M. et al. Haemotological, biochemical and serum electrolyte changes in non-pregnant Boer does inoculated with *Corynebacterium pseudotuberculosis* via various routes. **Journal of Agriculture and Veterinary Science**, v. 7, n. 10, p. 5-8, 2014.

PEREIRA, P. F. V. et al. Fatores de risco, etiologia e aspectos clínicos da mastite em ovelhas de corte no Paraná. **Pesquisa Veterinária Brasileira**, v. 34, n. 1, p. 1-10, 2014.

ROCHA, W. V. Perfil produtivo da pecuária e situação epidemiológica da tuberculose em fêmeas bovinas adultas no estado de Goiás. 2016. 105 f. **Tese (Doutorado) - Curso de Pós-graduação em Ciência Animal**, Universidade Federal de Goiás, 2016.

SELIM, A. M. et al. Risk factors associated with the seroprevalence of caseous lymphadenitis in sheep. **Comparative Clinical Patholog**, v. 30, n. 2, p. 285-291, 2021.

RUSSEL, A. Body condition scoring of sheep. **Farm Practice**, 1984. Disponível em: <doi.org/10.1136/inpract.6.3.91>.

SILVA, J. G. et al. Etiology of Mastitis in native goats and sheeps born and raised in Brazilian semi-arid biom. **Medicina Veterinária**, v. 7, n. 2, p. 26-31, 2013.

SILVEIRA, R. M. F. et al. Typification, characterization, and differentiation of sheep production systems in the Brazilian semiarid region. **NJAS: Impact In Agricultural And Life Sciences**, v. 93, n. 1, p. 48-73, 2021.

SOUZA, G. N. et al. Epidemiologia veterinária aplicada ao desenvolvimento de programas sanitários e controle de focos. 2018. **Circular técnica/Embrapa**. Juiz de Fora, MG Dezembro, 2018.

SOUZA, M. F. et al. Linfadenite caseosa em ovinos deslanados abatidos em um frigorífico da Paraíba. **Pesquisa Veterinária Brasileira**, v. 31, n. 3, p. 224-230, 2011.

UNANIAN, M. M.; FELICIANO SILVA, A. E. D.; PANT, K.P. Abscesses and caseous lymphadenitis in goats in tropical semi-arid Northeast Brazil. **Tropical Animal Health and Production**, v. 17, p. 57-62, 1985.

WAGNER, M. B. Medindo a ocorrência da doença: prevalência ou incidência? **Pediatrics journal**, v. 74, p. 154-162, 1998.

