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Mammary gland health of Santa Inês ewes at the drying and puerperium and evaluation of a dry-off terapy with gentamicin[1]

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ABSTRACT. Pereira P.F.V., Reway A.P., Félix A., Beutemmüller E.A., Pretto-Giordano L.G., Alfieri A.A., Lisbôa J.A.N. & Müller E.E. 2018. **Mammary gland health of Santa Inês ewes at the drying and puerperium and evaluation of a dry-off therapy with gentamicin.** *Pesquisa Veterinária Brasileira 38(12):2194-*2200. Departamento de Clínicas Veterinárias, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid PR-445 Km 380, Campus Universitário, Londrina, PR 86057-970, Brazil. E-mail: pri_fajardo@yahoo.com.br

Mastitis represents an important health problem for Santa Inês breed, causing losses to the producer, due to loss of ewes or the decrease in weight gain of lambs. The aim of this work was to assess the health of the mammary gland of Santa Inês ewes at the drying and puerperium and to investigate the efficacy of a dry-off therapy with gentamicin. In this study, 64 ewes were divided in a control group (GC) and treatment group (GT), and the health of the mammary gland was assessed at the drying and puerperium. The GT ewes received 250mg of gentamicin (Gentocin® DryCow/Schering-Plough, product indicated for use in dairy cows) in each mammary half. For diagnosis, clinical examination, California Mastitis Test, somatic cell count and milk culture was performed. In the GC, of the 45 (70.3%) healthy mammary halves at the drying, 12 developed subclinical mastitis and nine clinical mastitis at the puerperium. In the GT, among 51 (79.7%) healthy mammary halves at the drying, six developed subclinical mastitis and 11 clinical mastitis at the puerperium. No association was observed between treatment and the occurrence of mastitis at puerperium. Of the 19 (29.7%) mammary halves of the GC that presented subclinical mastitis at the drying, three remained with subclinical mastitis and five developed clinical mastitis at the puerperium. In the GT, of the 13 (20.3%) mammary halves that had subclinical mastitis at the drying, four remained with subclinical mastitis and four developed clinical mastitis. No association was observed between treatment and cure or persistence of mastitis at the puerperium. The main microorganisms isolated, at the drying and puerperium, from animals with subclinical or clinical mastitis were *Staphylococcus* spp., predominantly coagulase negative *Staphylococcus* (CSN). At the puerperium, 29 cases of clinical mastitis occurred, 19 with isolation, where 10 were CNS and six *S. aureus*. *Mannheimia haemolytica* was isolated in one case of subclinical mastitis and other of clinical mastitis. News protocols and different ways of handling at drying and at puerperium must be investigated.

INDEX TERMS: Mammary gland, health, ewes Santa Inês, dry-off terapy, sheep, mastitis, etiology, clinics.

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RESUMO.- [**Sanidade da glândula mamária de ovelhas Santa Inês na secagem e no puerpério e avaliação da terapia intramamária com gentamicina na secagem.**] A mastite é um problema sanitário importante em ovelhas da raça Santa Inês, ocasionando prejuízo ao produtor em virtude do descarte de matrizes e da queda no ganho de peso dos cordeiros. O objetivo deste trabalho foi avaliar a saúde da glândula mamária de ovelhas da raça Santa Inês na secagem e no puerpério e pesquisar a eficácia da terapia intramamária com gentamicina na secagem. Sessenta e quatro ovelhas foram divididas em grupos controle (GC) e tratamento (GT), cada um contendo 32 animais, e a saúde da glândula mamária avaliada na secagem e no puerpério. As ovelhas do GT receberam 250mg de gentamicina (Gentocin® Mastite Vaca Seca/ Schering-Plough Veterinária, produto indicado pela empresa para utilização em vacas de leite) em cada metade mamária. Para o diagnóstico, foram realizados exame físico da glândula mamária, California Mastitis Test, contagem de células somáticas e cultura do leite. No GC, das 45 (70,3%) metades mamárias sadias na secagem, 12 desenvolveram mastite subclínica e nove mastite clínica no puerpério. No GT, das 51 (79,7%) metades mamárias sadias na secagem, seis desenvolveram mastite subclínica e 11 mastite clínica no puerpério. Não houve associação entre o tratamento e a ocorrência de mastite no puerpério. Das 19 (29,7%) metades mamárias do GC que apresentaram mastite subclínica na secagem, três permaneceram com mastite subclínica e cinco desenvolveram mastite clínica no puerpério. No GT, das 13 (20,3%) metades mamárias com mastite subclínica na secagem, quatro permaneceram com mastite subclínica e quatro desenvolveram mastite clínica. Não houve associação entre o tratamento e a cura ou persistência da mastite no puerpério. Os principais micro-organismos isolados, na secagem e puerpério, de animais com mastite subclínica ou clínica foram *Staphylococcus* spp., com predominância de *Staphylococcus* Coagulase Negativa (SCN). No puerpério, ocorreram 29 casos de mastite clínica, sendo 19 com isolamento, 10 com SCN e seis com *S. aureus*. *Mannheimia haemolytica* foi isolado em um caso de mastite subclínica e um caso de mastite clínica. Novos protocolos e diferentes formas de manejo na secagem e no puerpério devem ser pesquisados

TERMOS DE INDEXAÇÃO: Glândula mamária, ovelhas Santa Inês, terapia intramamária, ovinos, mastite, etiologia, antibioticoterapia, clínica.

INTRODUCTION

Mastitis is responsible for high economic losses including a decrease in milk yield, in weight gain of lambs with eventual death, ewe culling off, and treatment expenses. However, the vast majority of studies regarding intramammary infections was performed in dairy sheep herds from Europe and North America, where the raising system employed is completely different from that used in Brazil (Menzies & Ramanoon 2001, Bergonier et al. 2003). The epidemiology and etiology of mastitis in meat ewes, including Santa Ines ewes, was studied recently in Brazil (Oliveira 2007, Blagitz et al. 2008, Coutinho et al. 2008a) and in the Paraná State (Pereira et al. 2014). Moreover, studies regarding intramammary therapy at dry-off stage in meat ewes are limited. This procedure is recommended in dairy cows, goats and ewes to treat any infection acquired during lactation period and to prevent new infections during dry and subsequent lactation periods (Chaffer et al. 2003). The therapy in dry cows is one of the most effective practices for mastitis control (Spanu et al. 2011). Because of the long period between the dry-off and the following parturition, the use of this procedure in meat sheep remains controversial (Bergonier et al. 2003). It is noteworthy that a specific intramammary antimicrobial product for small ruminants is not available in Brazil.

The present study aimed to evaluate the mammary gland health of Santa Ines ewes at dry-off and puerperium period and the efficacy of intramammary dry-off therapy with gentamicin.

MATERIALS AND METHODS

All experimental procedures and animal handling were perfomed after submission and approval by the Ethics Committe and Animal Experimentation of Universidade Estadual de Londrina (CEEA-UEL, No. 94/2010).

Selection of ewes. The study was performed on a farm located at the municipality of Ibiporã city (23°16'08" S, 51°02'52" W), north region of Paraná State, from September 2010 to September 2011. From a herd of 156 Santa Ines and Texel ewes (including rams, ewes, and lambs), 64 Santa Ines ewes were selected and kept in semi-intensive raising system. During the day, the sheep were kept on star grass (*Cynodon nlemfuensis*) pasture and at night were closed in shelters. Ewe selection was based on mammary gland inspection and palpation and also examination of mammary secretions (Grunert 1993). Only ewes without physical alterations in the mammary gland and milk were used. Ewes were individually identified.

Clinical examination and study design. The selected sheep, primiparous (n=4) or multiparous (n=60), were randomly allocated at to two groups (Control and Treatment, n=32 each). At 90±5 days of lactation, ewes from Control (CG) and Treatment (TG) groups were submitted to physical exam of the udders and milk samples were collected to perform California mastitis test (CMT), somatic cell count (SCC), microbiological culture, and antibiogram. For milk sample collection, the ewes were previously kept separate from the lambs for approximately six hours. Afterwards, the milk of the females from CG and TG were completely drained by hand milking. Ewes from TG received 250mg of gentamicin (Gentocin® Mastite Dry cow, Schering-Plough Veterinaria, originally indicated for cows) in each mammary half. After dry-off, the sheep were not milked again. The ewes were kept in the routine semi-intensive raising system of the farm.

Approximately 7 days before parturition, ewes were confined in collective pens for a better monitoring. The same evaluations performed at dry-off were conducted at puerperium (7 to 10 days after parturition).

In the evaluation of the mammary gland sanitary conditions, the mammary halves were classified according to its clinical conditions, SCC and milk microbiological culture. The characterization of subclinical mastitis used 500,000 cells/mL as threshold, in accordance with Berthelot et al. (2006). Thus, at the dry-off, the mammary halves were classified as healthy or with subclinical mastitis; and at the puerperium they were classified as healthy, with subclinical mastitis or with clinical mastitis. In the mammary halves classified as healthy, the milk was normal, SCC was lower than 500,000 cell/mL, and microbiological culture was negative. In those ones classified as with subclinical mastitis, the milk sample had SCC ≥500,000 cells/mL and/or was positive in microbiological culture. The mammary halves with clinical mastitis had evident signs of inflammation (heat,

redness, tumor, pain, partial/total function loss, characterized by the absence of milk secretion) with or without bacterial isolation in microbiological culture.

Diagnostic methods. The diagnosis of clinical mastitis was performed by inspection and palpation of the udder. The mammary parenchyma was evaluated for the presence of nodules, diffuse hardness areas, and fibrosis. Moreover, the macroscopic characteristics of the milk (color, consistency, presence of lumps, pus, and blood) were evaluated, according to Grunert (1993).

Presumptive diagnosis of subclinical mastitis was performed using CMT (Schalm & Noorlander 1957), considering negative (-) and positive (score 1+, 2+, 3+) reactions.

Milk samples collected for SCC analysis were performed in vials containing bronopol. The vials were packed in boxes and sent to the Laboratório Centralizado de Análise de Leite da Associação Paranaense de Criadores de Bovinos da Raça Holandesa (Curitiba/PR), where they were analyzed by flow cytometer (SOMACOUNT 500, Bentley Instruments Inc).

Milk sample collection for microbiological culture and identification was performed according to the instructions of the National Mastitis Council (NMC 2004), preconized for bovine. Milk samples were collected after hand sanitizing, washing of the teat with water and soap, drying, discard of the first stream of milk, immersion in chlorine solution (750ppm), cleaning with alcohol (70ºGL), and drying with paper towel. The milk was collected in sterile screw cap glass vials, and transported refrigerated (4-8ºC) in isotherm container to the Laboratório de Microbiologia do Departamento de Medicina Veterinária Preventiva (DMVP-UEL). At the laboratory, samples were seeded in petri dishes with agar nutrient media (Himedia®) with 5% of ovine blood and MacConkey agar (Himedia®). They were incubated at 37±1ºC, and the readings were performed 24, 48, 72, and 96 hours later. The isolated microrganisms were identified based on morphological, Gram stain, biochemical and culture characteristics (Carter & Cole Junior 1990, Quinn et al. 1994, NMC 2004).

Staphylococcus spp. were submitted to coagulase test and identified by API-STAPH system (Bio Mérieux®, France). *Streptoccus* spp. were classified in groups by SLIDEX STREPTO-KIT (Bio Mériux®, France) and identified by API-STREP (Bio Mériux®, France).

The diagnosis of *Mannheimia haemolytica* was based on the morphological characteristics and Gram stain. For specie confirmation, the colonies were submitted to polymerase chain reaction (PCR) using primers from the intergenic region between artJ and lktC at the Laboratório de Virologia of DMVP-UEL, according to the technique previously described (Angen et al. 2009). The amplified products were sequenced and the identity was compared with sequence from public data bases.

Antibiogram was performed using disc diffusion method (Bauer et al. 1966) in Mueller Hinton agar (Himedia®) and discs (Laborclin®) impregnated with the following antimicrobial agents: Amoxicillin (AMO - 10µg), Ampicillin (AMP - 10µg), Ceftiofur (CFT - 30µg), Enrofloxacin (ENR - 5µg), Streptomycin (EST - 300µg), Gentamicin (GEN - 10µg), Neomicin (NEO - 30µg), Oxacillin (OXA - 10µg), Penicillin G (PEN - 10UI), Sulfonamide (SUF - 25µg), and Tetracicline (TET - 30µg).

Statistical analysis. Prevalence of mastitis in control and treatment groups was compared by contingency tables and qui-square test. Quantitative variables were compared by Mann-Whitney and t tests. A 5% significance level was used.

Kappa coefficient (Cohen 1960) was calculated to evaluate the agreement between SCC and microbiological culture. Agreement was classified as low (values ranging from 0.01 to 0.2), medium (0.21 to 0.4), moderate (0.41 to 0.6), substantial (0.61 to 0.8), and high (0.81 to 1.0), according to Landis & Koch (1977).

RESULTS AND DISCUSSION

Table 1 shows the results of CMT, SCC and culture of milk samples collected at dry-off and puerperium period, not considering the intramammary treatment. At dry-off, 43 (34%) of the mammary halves had CMT ≥1+, 24 (19%) had SCC ≥500.000 cells/mL, and 32 (25%) were positive in microbiological culture. At puerperium, 46 (36%) of the mammary halves had CMT ≥1+, 37 (29%) had CCS ≥500.000 cells/mL, and 33 (26%) were positive in microbiological culture. In this phase, 29 cases of clinical mastitis and 25 cases of subclinical mastitis were observed; however, in 12 cases of subclinical mastitis, as well as in one case of clinical mastitis, no microrganisms were isolated. In seven mammary halves, milk sample collection was not performed because of the severe inflammation signs and diffuse hardness of the mammary parenchyma, leading to an absence of milk production. Pereira et al. (2014) also reported clinical mastitis with diffuse hardness of the udder and absence of milk production in Santa Inês ewes. In a dairy sheep herd from Jordan, microorganisms were isolated in 39.1% of the 1,210 milk samples used in the study (Lafi 2006).

In the present study, a high number of clinical mastitis cases was observed after parturition (29/64). It was reported that in 80% of the 31 Santa Ines sheep herds studied by Oliveira (2007), the occurrence of clinical mastitis was higher during puperperium period. Similarly, an epidemiological survey in the North region of Parana State demonstrated that in 69,2% of the meat sheep flocks studied most cases of clinical mastitis occurred after parturition (Pereira et al. 2014).

Table 1. California Mastitis Test (CMT), somatic cell count (SCC) and milk culture results of 128 mammary halves of Santa Ines ewes at dry-off and puerperium period, Londrina/PR

Lactation period	No.	CMT			SCC (x10^3 cells/mL)			Microbiological culture		
		<1+	≥1+	NP*	< 500	≥500	NP*	Negative	Positive	NP**
Dry-off	128	85 (66%)	43 (34%)	0	88 (69%)	24 (19%)	16 (12%)	96 (75%)	32 (25%)	0
Puerperium	128	78 (61%)	46 (36%)	4 (3%)	72 (56%)	37 (29%)	19 (15%)	89 (69%)	32 (25%)	7 (6%)

*NP = Exam not performed due to reduced or absent milk production, **NP = Exam not performed due to absent milk production as the result of clinical mastitis.

With a threshold of 500,000 cells/mL, the kappa coeficient between SCC and milk culture results was 0.43 (P<0.001), indicating a moderate agreement between these two tests. Sensitivity and specificity of SCC was 62% and 83%, respectively. Previous studies in dairy flocks used different threshold values for SCC. A study in Slovenia with 251 dairy sheep used 250,000 cells/mL as threshold (Pengov 2001). In a study in Jordan, the threshold used was 1,000,000 cells/mL (Lafi 2006). When a threshold of 500,000 cells/mL was used the sensitivity and specificity observed was 73% and 82%, respectively (Berthelot et al. 2006).

Health conditions of the mammary gland of ewes from CG and TG at dry-off and puerperium period are shown in Table 2. An association between the treatment and the occurrence of mastitis during puerperium was not observed (P=0.261), i.e., the use of intramammary antibiotic at dry-off did not prevent the occurrence of subclinical or clinical mastitis in this study. A study with 245 dairy sheep showed that the intramammary therapy at dry-off did not affect the occurrence of intramammary infections at parturition, although the SCC significantly decreased in the treated group (Spanu et al. 2011). In contrast, a significant decreased of new cases of mastitis in the following lactation was observed after therapy at dry-off in several studies (Bergonier & Berthelot 2003, Gonzalo et al. 2004, Linage & Gonzalo 2008).

Also, there was no association between the treatment and the healing or persistency of the mastitis during puerperium (P=0.472), i.e., the use of intramammary antibiotic did not eradicate the infection and did not prevent the occurrence of subclinical or clinical mastitis. Healing rates between 65% and 98% was reported after the treatment with intramammary antibiotics (Bergonier & Berthelot 2003). A study with 85 dairy sheep in Israel reported higher healing rates in sheep treated with intramammary antibiotic at dry-off, although the rate of new infections was not different between treated and control group (Chaffer et al. 2003). A healing rate of 100% was reported after the treatment with intramammary cefalonium of sheep with subclinical mastitis at dry-off (Coutinho et al. 2008b).

Several reasons could explain why the intramammary therapy with gentamicin at dry-off fails to prevent new infections or heal previous infections. In this experiment, a high number of new infections also occurred during puerperium, most of them clinical form. The long dry period (ranging from 5 to 10 months) observed in the present study could be one explanation. In the farm studied, breeding season or reproductive management was not employed because having parturition spread all over the year was interesting to the owner. This type of management is common in several farms located in the North region of Paraná State (Pereira et al. 2014). The dry period of sheep can be as long as six months or more, and it is considered a limiting factor to the use of intramammary antibiotics at dry-off, since most of the drugs available not remain active throughout this period (Bergonier et al. 2003, Chaffer et al. 2003). A study in Spain with 229 dairy sheep with a shorter dry period (109 days in average) reported that the prevalence of intramammary infection decreased significantly from 48% at dry-off to 13% at parturition in those animals treated with an association of antibiotics at dry-off (Linage & Gonzalo 2008). However, in this same experiment, no difference in the prevalence of infections was observed in control group. The parenteral administration of tilmicosina one month before parturition did not reduce the cases of clinical mastitis, but attenuated the palpable abnormalities in the udder (Croft et al. 2000).

Table 2. Health conditions of the mammary halves at dry-off and puerperium period of 64 Santa Ines sheep from control and treatment groups, Londrina/PR

Groups		Dry-off N	Puerperium		
			Healthy	Subclinical mastitis	Clinical mastitis
Control group	Healthy	45	24 (53.3%)	12 (26.7%)	9 (20.0%)
	Subclinical mastitis	19	11 (57.9%)	3 (15.8%)	5 (26.3%)
Subtotal		64	35	15	14
Treatment group*	Healthy	51	34 (66.7%)	6 (11.8%)	11 (21.5%)
	Subclinical mastitis	13	5 (38.4%)	4 (30.8%)	4 (30.8%)
Subtotal		64	39	10	15
TOTAL		128	74	25	29

*Intramammary administration of gentamicin at dry-off.

The confinement of the ewes one week before parturition to allow a better monitoring could favor the occurrence of mastitis during puerperium. The ewes were confined in collective pens and cleaning was not daily performed. The accumulation of waste in the sheepfold and keeping healthy sheep together with sheep affected by mastitis in the same pen can favor the transmission of the contagious agent by lamb suckling, contributing for new infections. An epidemiological study in the North region of Parana State demonstrated that only 22% of the farmers performed the cleaning of the sheepfold daily and the rest of them performed the cleaning at irregular intervals, resulting in accumulation of organic waste (Pereira et al. 2014). In this same study, the intensive management system was identified as a risk factor for mastitis. Marogna et al. (2010) reported that in herds affected by chronic mastitis, the unsatisfactory hygienic conditions and the overcrowding of the sheepfolds have favored the development of microorganism and infection of the mammary gland.

Another factor to be considered as the cause of the ineffectiveness of preventive antibiotic therapy at dry-off is the decreased activity of the phagocytes. These cells play a key role in the elimination of the microorganisms that cause mastitis (Dosogne et al. 1998). Previous studies in dairy cows showed that different antibiotics indicated to be used at dry-off had a negative influence over the phagocytic activity of the somatic cells (Paape et al. 1996, Batista et al. 2006). This same effect was observed in goat milk phagocytes (Benesi et al. 2010). The effect of several intramammary antibiotics over phagocytosis was tested in vitro, and was observed that gentamicin lead to the lowest level of phagocytosis.

The effectiveness of antibiotic therapy at dry-off is controversial due to high rates of self-healing in sheep and goat, regardless the administration of intramammary antibiotic at dry-off (Fox et al. 1992). In the present study (Table 2), from 19 mammary halves with subclinical mastitis at dry-off in CG, 11 (57.9%) were healthy after parturition. Contreras et al. (2007) reported that healing rates can range

from 20% to 60% and intramammary therapy at dry-off was recommended only in herds with high prevalence of mastitis.

The microganisms isolated from the milk samples obtained from mammary halves at dry-off and puerperuim period are shown in Table 3. The most prevalent microorganism at dry-off and puerperium period in both groups was *Staphylococcus* spp. (87.7%) and the different species isolated are shown in Table 4. These results corroborate with previous studies. In 251 dairy ewes, *Staphylococcus* spp. was isolated in 99 (76.2%) of 130 milk samples with positive result in culture (Pengov 2001). The genus *Staphylococcus* is the main etiological agent of mastitis in dairy sheep; moreover, it was reported that coagulase negative staphylococci are responsible for 25% to 93% of the infections, and *S. aureus* are responsible for 3% to 37% (Bergonier et al. 2003).

Regarding coagulase positive staphylococci, were identified 16 *S. aureus*, six from clinical mastitis and 10 from subclinical mastitis, and three samples of *S. intermedius* isolated from subclinical infections. *S. aureus* is the etiological agent most commonly isolated from clinical mastitis in ewes (Kirk et al. 1996, Jones & Watkins 2000, Bergonier et al. 2003).

Species of coagulase negative staphylococci were more prevalent at dry-off and also at puerperium period, specially *S. chromogenes* and *S. xylosus*. Previous study reported that 45% of the microrganisms isolated from sheep with mastitis were coagulase negative staphylococci, and the most prevalent were *S. xylosus*, *S. chromogenes* and *S. epidermidis* (Spanu et al. 2011). A study with 3,758 milk samples showed that 87.5% of the isolated were coagulase negative staphylococci and only 0.8% were *S. aureus* (Berthelot et al. 2006). In Santa Ines ewes, 40% of the milk samples were positive for *S. aureus* and 20% for coagulase negative staphylococci (Guarana et al. 2011). In general, the most frequently coagulase negative staphylococci isolated from sheep with mastitis are: *S. epidermidis*, *S. chromogenes*, *S. xylosus*, *S. simulans* e *S. hyicus* (Pengov 2001, Bergonier et al. 2003, Spanu et al. 2011). Pereira et al. (2014) reported CNS as the most prevalent microorganism isolated form clinical mastitis cases in meat ewes.

In sheep sampled, the median of SCC of milk sample in which were isolated coagulase positive and negative staphylococci was 568,000 and 436,000, respectively; and from 20 cases of mastitis caused by coagulase negative staphylococci in the puerperium, 10 presented as clinical mastitis. In cows,

Table 3. Microrganims isolated in milk samples of Santa Ines sheep in control group and intramammary gentamicin treated group at dry-off and puperperium period, Londrina/PR

Microrganism	Control group		Treatment group		Total
	Dry-off	Puerperium	Dry-off	Puerperium	
CPS*	5	3	6	5	19 (29.7%)
CNS**	11	12	5	8	36 (56%)
Streptococcus bovis	0	0	1	0	1 (1.6%)
Streptococcus dysgalactie	0	1	0	0	1 (1.6%)
Streptococcus spp. Group D	1	0	0	0	1 (1.6%)
Streptococcus spp. Group G	0	0	1	0	1 (1.6%)
Streptococcus spp.	1	0	0	0	1 (1.6%)
Aerococcus spp.	1	0	0	0	1 (1.6%)
Klebsiella pneumoniae	0	0	0	1	1 (1.6%)
Mannheimia haemolytica	0	1	0	1	2 (3.1%)
Total of positive samples	19	17	13	15	64

*CPS = coagulase positive *Staphylococcus*, **CNS = coagulase negative *Staphylococcus*.

Table 4. Specie identification in 55 samples collected from Santa Ines sheep at dry-off and puerperium period where *Staphylococcus* was isolated, Londrina/PR

Specie	Dry-off		Puerperium		Total	
	N	%	N	%	N	%
CPS*	11	40.8	8	28.6	19	34.5
S. aureus	10	37.0	6	21.4	16	29.0
S. intermedius	1	3.8	2	7.2	3	5.5
CNS**	16	59.2	20	71.4	36	65.5
S. chromogenes	3	11.1	5	17.9	8	14.5
S. hyicus	4	14.8	2	7.2	6	11.0
S. sciuri	0		2	7.2	2	3.6
S. simulans	2	7.4	4	14.2	6	11.0
S. xylosus	4	14.8	3	10.7	7	12.7
Not identified	3	11.1	4	14.2	7	12.7
TOTAL	25	100	28	100	55	100

*CPS = coagulase positive *Staphylococcus*, **CNS = coagulase negative *Staphylococcus*

coagulase negative staphylococci are considered agents of low pathogenicity and responsible for most cases of subclinical mastitis (Contreras et al. 2007, Arsenault et al. 2008). In contrast, others authors considered coagulase negative staphylococci in ewes as responsible for the significant increase of SCC and as an important etiological agent of clinical mastitis (Fthenakis & Jones 1990, Pengov 2001).

Streptococcus spp. were isolated in five milk samples (7.8%), four at the dry-off and one in the puerperium period. This genus was the second most prevalent in a previous study (Pengov 2001). In milk sample collected from Santa Ines sheep, within 10 to 45 days after parturition, 12.1% of the isolated microrganisms were classified as *Streptococcus* spp. (Coutinho et al. 2008b). In Brazilian Northeast, 15.9% of the samples collected from Santa Ines sheep were positive for *Streptococcus* spp. (Guarana et al. 2011).

M. haemolytica was isolated from two milk samples collected during puerperium, one sample from a subclinical mastitis case (SCC of 1,342,000 cells/mL) and another from an acute clinical mastitis case, with diffuse hardness of the mammary parenchyma and reddish color milk secretion. This microrganism is considered one of the most frequent etiological agent of clinical mastitis in meat ewes. Saliva and respiratory secretions of the lambs are the main infection source of the pathogen in sheep (Kirk & Glenn 1996, Menzies & Ramanoon 2001, Omaleki et al. 2011). In Brazil, reports of the involvement of *M. haemolytica* in intramammary infections in ovine are limited. Pereira et al. (2014) isolated *M. haemolyitca* in two cases of acute mastitis in meat ewes of Paraná state. In a study of 78 animals located in Northeast region of Brazil, *M. haemolyitca* was isolated in only one sample collected from a clinical mastitis case (Santos 2008). In anothers Brazilian studies about the mastitis etiology in ovine, isolation of *M. haemolyitca* was not reported (Oliveira 2006, 2007, Blagitz et al. 2008, Coutinho et al. 2008a).

Gentamicin, antibiotic used in the present study, had sensitivity of 90.6% and 81.2% face to microrganisms isolated at dry-off and puerperium, respectively (Table 5). Other studies with Santa Ines sheep, demonstrated that gentamicin was considered one of the best drugs to eliminate the microrganisms isolated from milk samples (Domingues et al. 2006, Coutinho et al. 2008a, Guaraná et al. 2011). Previous studies reported that the microorganism isolated from Santa Ines sheep with mastitis had lower sensitivity to streptomycin, kanamycin and tetraciclin (Almeida 2007, Guarana et al. 2011). The in vitro effectiveness of the antibiotics to eliminate the etiological mastitis agent in ovine in the present study can be attributed to the sporadic use of this drug in ovine industry, unlike what is observed in dairy cattle. The efficacy of other drugs should be tested in the drying off therapy in meat ewes, as well as the appropriate time to perform them.

Table 5. Antibiotic sensitivity profile of the microrganisms isolated from ovine clinical and subclinical mastitis at dry-off and puperperium period, Londrina/PR

Antibiotic	Dry-off		Puerperium	
	Sensitivity (%)	Resistance (%)	Sensitivity (%)	Resistance (%)
Amoxacillin	32 (100%)	0	31 (96.9%)	1 (3.1%)
Ampicillin	23 (72.0%)	9 (28.0%)	27 (84.4%)	5 (15.6%)
Ceftiofur	28 (87.5%)	4 (12.5%)	30 (93.7%)	2 (6.3%)
Enrofloxacin	22 (68.7%)	10 (31.3%)	29 (90.6%)	3 (9.4%)
Streptomycin	18 (56.2%)	14 (43.8%)	22 (68.7%)	10 (31.3%)
Gentamicin	29 (90.6%)	3 (9.40%)	26 (81.2%)	6 (18.8%)
Neomicin	19 (59.4%)	13 (40.6%)	26 (81.2%)	6 (18.8%)
Oxacilin	28 (87.5%)	4 (12.5%)	26 (81.2%)	6 (18.8%)
Penicillin G	20 (62.5%)	12 (37.5%)	19 (59.4%)	13 (40.6%)
Sulfonamide	25 (78.0%)	7 (22.0%)	29 (90.6%)	3 (9.4%)
Tetraciclin	25 (78.0%)	7 (22.0%)	23 (72.0%)	9 (28.0%)

CONCLUSIONS

Considering the experimental conditions, one may conclude that intramammary administration of gentamicin at dry-off did not prevent new infections and also did not heal the previous subclinical mastitis.

Moreover, a high prevalence of mastitis was observed during puerperium, mainly clinical mastitis form.

Coagulase negative *Staphylococcus* was mostly isolated, followed by coagulase positive *Staphylococcus*, both in clinical and subclinical mastitis, corroborating with the predominance of this group of bacteria as the etiological agent of mastitis in ovine.

The low occurrence of *Mannheimia haemolytica* indicates the reduced influence of this microrganism for mastitis etiology in the flock studied.

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