

OCCURRENCE OF ETIOLOGIC AGENTS CAUSING SUBCLINICAL MASTITIS IN MORADA NOVA AND SANTA INES EWES¹

OCORRÊNCIA DOS AGENTES ETIOLÓGICOS CAUSADORES DE MASTITE SUBCLÍNICA EM OVELHAS DAS RAÇAS MORADA NOVA E SANTA INÊS

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SUMMARY

This study aimed to determine the occurrence of the etiologic agents of subclinical mastitis in Santa Inês and Morada Nova ewes and their susceptibility to developing the disease when submitted to the same management conditions. We analyzed 250 mammary glands of 130 Santa Inês ewes and 143 mammary glands of 77 Morada Nova ewes. The California Mastitis Test, somatic cell counts and microbiological analysis were performed at the moment of drying off. The occurrences of subclinical mastitis in different breeds were analyzed using the chi-square test by adjusting the values according to the Yates continuity correction. The infectious subclinical mastitis was present in 33.1% and 35.1% of Santa Inês and Morada Nova ewes, respectively. Among the evaluated mammary glands of Santa Inês ewes, 20.4% had subclinical mastitis with the following infectious etiology: Coagulase-negative *Staphylococci* (CNS) (46%), Coliforms (22.0%), *Streptococcus* spp. (12.0%), *Corynebacterium* spp. (6%), *Micrococcus* spp. (6.0%), *Staphylococcus aureus* (2.0%), Coagulase-positive *Staphylococci* (2.0%) and CNS and *Streptococcus* spp. (4.0%) mixed infection. Of the Morada Nova ewe mammary glands evaluated, 21% had subclinical mastitis with the following etiologic agents and their occurrences: CNS (56.7%), Coliforms (13.3%), *Corynebacterium* spp. (10.0%), *Staphylococcus aureus* (10.0%), *Micrococcus* spp. (6.7%) and *Streptococcus* spp. (3.3%). The CNS can be considered the most important etiological agent of subclinical mastitis in sheep. Santa Inês and Morada Nova ewes have the same chances of developing subclinical mastitis when subjected to the same management system.

KEY-WORDS: Morada Nova. Santa Inês. Subclinical mastitis.

RESUMO

Este trabalho objetivou determinar a ocorrência dos agentes etiológicos da mastite subclínica em ovelhas das raças Santa Inês e Morada Nova e as susceptibilidades das duas raças em desenvolver a doença quando submetidas às mesmas condições de manejo. Analisou-se 250 mamas de 130 ovelhas da raça Santa Inês e 143 mamas de 77 ovelhas da raça Morada Nova. No momento da secagem, realizaram-se o *California Mastitis Test*, contagem de células somáticas e análises microbiológicas. As ocorrências da mastite subclínica nas diferentes raças foram analisadas utilizando o teste de qui-quadrado ajustando os valores de acordo com a correção de continuidade de Yates. As ocorrências de animais com mastite subclínica infecciosa foram de 33,1% e de 35,1% nas ovelhas Santa Inês e Morada Nova, respectivamente. Dentre as mamas avaliadas das ovelhas Santa Inês, 20,4% apresentaram mastite subclínica com a seguinte etiologia infecciosa: *Staphylococcus* coagulase-negativos (SCN) (46%), Coliformes (22%), *Streptococcus* spp. (12%), *Corynebacterium* spp. (6%), *Micrococcus* spp. (6%), *Staphylococcus aureus* (2%), *Staphylococcus* coagulase-positivos (2%) e infecção mista de SCN e *Streptococcus* spp. (4%). Das mamas avaliadas das ovelhas Morada Nova, 21% apresentaram mastite subclínica com os seguintes agentes etiológicos e respectivas ocorrências: SCN (56,7%), Coliformes (13,3%), *Corynebacterium* spp. (10,0%), *Staphylococcus aureus* (10,0%), *Micrococcus* spp. (6,7%) e *Streptococcus* spp. (3,3%). Os SCN podem ser considerados os mais importantes agentes etiológicos da mastite subclínica ovina. Ovelhas das raças Santa Inês e Morada Nova apresentam as mesmas chances de desenvolver mastites subclínicas quando submetidas ao mesmo sistema de manejo.

PALAVRAS-CHAVE: Mastite subclínica. Morada Nova. Santa Inês.

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INTRODUCTION

Several health problems are relevant in sheep farming. Mastitis is the inflammation of the mammary gland, usually of infectious origin and considered limiting for the exploration activity due to the consequent economic impacts (SANTOS et al., 2007). Among the losses, mortality, lower performance and breeder disposal are highlighted because they affect directly meat production efficiency. Thus, efforts are needed to explore solutions to control disease in livestock (VERÍSSIMO et al., 2010).

Mastitis can be classified as clinical or subclinical manifestation of the symptoms. The clinical syndrome has variable signs depending on inflammation severity while in subclinical mastitis signs are not visually detectable and can only be detected by complementary tests (MAROGNA et al., 2010). Subclinical mastitis is characterized by quantitative and qualitative changes in milk, especially the increasing number of somatic cells (BERGONIER & BERTHELOT, 2003). There is an association between clinical mastitis appearance and subclinical mastitis as sheep with a previous history of the disease have higher chances of developing mastitis from the following delivery (WAAGE & VATN, 2008).

There is a lack of studies addressing the diagnosis of ovine mastitis, especially breeds suitable for meat production (NUNES et al., 2008). Even sheep reared on extensive pasture systems may have a high occurrence of mastitis (AL-MAJALI & JAWABREH, 2003). Among the preventive measures against mastitis, the use of antimicrobials during drying is widely recognized as this disease's main preventive and curative method, but this management type is hardly known for sheep. For a successful antibiotic therapy, the dose should be the minimum necessary in order to avoid its side effects (SAHA et al., 2007).

Given the importance of identifying the etiologic agents of subclinical mastitis in meat sheep herds in order to adopt disease control measures, this study aims at determining the occurrence of infectious agents in Santa Inês and Morada Nova sheep at the time of drying and their susceptibility to developing the disease when subjected to the same management conditions.

MATERIAL AND METHODS

Site, time and experimental animals

The study was conducted in a herd located in São Carlos-SP between June 2012 and March 2013. We analyzed a total of 393 mammary glands, of which 250 from 130 Santa Inês ewes and 143 from 77 Morada Nova ewes. The sheep were reared under the same semi-intensive management system. All animals were housed in Coast-cross and *Brachiaria decumbens* grass paddocks and supplemented with daily supply of corn silage, mineral and water *ad libitum* throughout the experimental period. The animals were supplemented with concentrated feed in the flushing

system in the last month of pregnancy, and creep-feeding the newborn lambs. The reproductive management consisted of natural mating with breeders of the same breed. The places where the animals slept or were sheltered from the rain were cleaned constantly; however, stool and humidity were always present.

Milk sampling

An average 89 days after calving, the lambs were separated from their mothers for 12 hours prior to sampling. Before sampling, physical examination of the mammary gland was performed, and the teat sphincter was disinfected with 70% isopropyl alcohol. The milker washed his hands with soap and water before collecting the milk samples. A 2-mL milk sample was collected from each gland for immediate realization of the California Mastitis Test (CMT), a 60-mL milk sample from each gland was placed in a plastic container containing the preservative bronopol, transferred to the lab to determine the somatic cell counts (SCC), electronically. Another two 5-mL milk samples from each breast of the ewes were collected and placed in sterile glass tubes intended for microbiological testing. There were no differences in climate at the time of sample collection of different species.

California Mastitis Test (CMT) and somatic cell count (SCC)

The CMT testing of milk samples was performed according to Schalm and Noorlander (1957), after mixing the 2 mL milk of each gland with the same amount of CMT reagent (neutral anionic surfactant detergent). After mild homogenization, the samples were classified according to the degree of precipitation and gel formation in four different scores: negative, weakly positive (1+), positive (2+) or strongly positive (3+).

The samples for SCC testing were transferred to the laboratory of Clínica do Leite, located in Piracicaba, SP. The counts were performed using the electronic device Somacount 300 (Bentley Instruments®).

Microbiological diagnosis

The microbiological diagnosis of subclinical mastitis was performed in the laboratories of Embrapa Pecuária Sudeste, after sowing 0.01 mL of milk on Petri dishes containing 5% defibrinated sheep blood agar, incubated under aerobic conditions at 37°C for 72 hours, with readings taken from the plates every 24 hours. Macroscopic characteristics of the colonies, as morphology, pigment production and hemolysis were observed, with identification of micro-organisms according to the morpho-staining, biochemical and cultivation characteristics following Koneman et al. (2001). The microbiological tests results were interpreted according to Harmon et al. (1990).

Determination of infectious subclinical mastitis

The mammary glands that did not present detectable clinical abnormalities, but had positive scores either to CMT or $SCC > 2.5 \times 10^5$ cells.mL⁻¹.milk (PENGOV, 2001) were considered positive for infectious mastitis. However, given the characteristics of the etiologic agents isolated from the mammary glands that had SCC values between 70,794 and 158,489 cells.mL⁻¹.milk, with isolates of Coagulase-negative *Staphylococci* resistant to *novobiocin*, *Micrococcus* spp. or *Corynebacterium* spp. were also classified as positive to infectious mastitis (ARIZNABARRETA et al., 2002).

Statistical Analysis

Predisposition to ovine subclinical mastitis of Santa Inês and Morada Nova breeds was analyzed using the chi-square test with a confidence interval of 95% (95% CI). The significant chi-square values close to those tabulated values were adjusted according to the Yates correction for continuity. Data were analyzed using EpiInfo version 7.1.1.14 software (*Epi Info*TM 7.1.1.14, 2013). Mastitis relative risk (RR) for the studied breeds was calculated.

Analysis of the ethics committee

The use of animals in this study was reviewed and approved by the Ethics Committee for Animal Use in Research of Embrapa Pecuária Sudeste in the submission of the project.

RESULTS AND DISCUSSION

No significant difference (RR=1.06, CI: 0.71 to 1.56; P=0.89) was observed regarding susceptibility to subclinical mastitis of ewes from Santa Inês and Morada Nova breeds. Positive rates for infectious mastitis in Santa Inês and Morada Nova breeds were 33.1% and 35.1%, respectively.

Of the total of 393 mammary glands examined in this study, 80 (20.4%) were positive for infectious mastitis. Coagulase-negative *Staphylococci* was the predominant agent (52.4% of cases) in subclinical mammary infections. Table 1 shows the infectious agents occurrence detected in isolated or mixed infections.

Of the 250 mammary glands of Santa Inês sheep evaluated, 20% (50 glands) were positive and the etiologic agents' occurrence is shown in Table 2. The results reported in this study are lower than the subclinical mastitis occurrence obtained by Morais et al. (2011) (58.7%), similar to those of Guarana et al. (2011) (occurrences between 23.53% and 35.29%) and higher than those of Silva et al. (2010) (7.4%).

Of the 143 mammary glands of Morada Nova sheep, 21% (30 breasts) were positive and the etiologic agents' frequency distribution is described in Table 3.

Corroborating the results of this study, Silva et al. (2010), Guarana et al. (2011), Domingues et al. (2006) and Coutinho et al. (2006) also reported the Coagulase-negative *Staphylococci* as the infectious agents in most subclinical infections of Santa Inês

sheep with occurrences of 26.9%, 65.9%, 67.9% and 57.6%, respectively.

In the state of Pará, Silva et al. (2010) submitted Santa Inês ewes to CMT and microbiological testing, and isolated, in addition to the CNS, *Staphylococcus aureus* in 15.4%, *Streptococcus* spp. in 7.69%, *Escherichia coli* in 7.69% and *Citrobacter freundii* in 11.5% of the cases.

Coutinho et al. (2006) analyzed 124 milk samples from 62 Santa Inês ewes at the time of drying, 26.6% were microbiologically positive. In addition to CNS, *Staphylococcus aureus*, *Micrococcus* spp., *Streptococcus a hemolytic* and *Streptococcus agalactiae* were isolated in, respectively, 15.2%, 15.2%, 9% and 3% of the mammary gland.

Domingues et al. (2006) reported that the most frequent associations of micro-organisms in the analyzed milk samples were between *Staphylococcus* spp. and *Streptococcus* spp. (33.4%) and between *Staphylococcus aureus* sp and *Corynebacterium* spp. (28.6%). In this work, only the association between *Streptococcus* spp. and *Staphylococcus* spp. was observed in subclinical infections of Santa Inês sheep (4% of cases) while mixed infections were not observed in Morada Nova sheep with subclinical mastitis.

Morais et al. (2011) reported that the practice of not fully milking the mothers at the end of lactation is a relevant factor for predisposition to the disease. According to Pengov and Kirbis (2009), administration of antimicrobial agents in the drying phase of the sheep has two objectives: to eliminate existing subclinical intramammary infections, which can cause recrudescence of clinical disease and prevent new infections during the dry period, when sheep are particularly susceptible.

The treatment during sheep dry phase can also be effective and implemented in herds with high prevalence of mastitis. Moreover, issues such as the appropriate cannula for the species and dosage of antibiotics should be addressed by further studies (CHAFFER et al., 2003).

CONCLUSION

Various bacteria can be present in subclinical mammary infections of meat sheep. The Coagulase-negative *Staphylococci* can be considered the most important etiologic agent. Santa Inês and Morada Nova sheep have the same chances of developing subclinical mastitis when subjected to the same management system. Specific measures to treat and prevent subclinical mastitis of meat sheep should be introduced in order to reduce the resulting losses.

Table 1 - Occurrence of etiologic agents in single and mixed mammary infections in meat sheep.

Microorganism	Occurrence (%/no.)
CNS	50.0 / 40
Coliforms	18.75 / 15
<i>Streptococcus</i> spp.	8.75 / 7
<i>Corynebacterium</i> spp.	7.50 / 6
<i>Micrococcus</i> spp.	6.25 / 5
<i>Staphylococcus aureus</i>	5.0 / 4
CPS	1.25 / 1
NCS + <i>Streptococcus</i> spp.	2.5 / 2
Total	100 / 80

CNS = Coagulase-negative *Staphylococci*CPS = Coagulase-positive *Staphylococci***Table 2** - Occurrence of etiologic agents in single or mixed in mammary infections of Santa Ines sheep.

Microorganism	Occurrence (%/no.)
CNS	46 / 23
Coliforms	22 / 11
<i>Streptococcus</i> spp.	12 / 6
<i>Corynebacterium</i> spp.	6 / 3
<i>Micrococcus</i> spp.	6 / 3
<i>Staphylococcus aureus</i>	2 / 1
CPS	2 / 1
CNS + <i>Streptococcus</i> spp.	4 / 2
Total	100 / 50

CNS = Coagulase-negative *Staphylococci*CPS = Coagulase-positive *Staphylococci***Table 3** - Results of etiologic agents in single or mixed in mammary infections of Morada Nova sheep.

Microorganism	Occurrence (%/no.)
CNS	56,7 / 17
Coliforms	13,3 / 4
<i>Corynebacterium</i> spp.	10 / 3
<i>Staphylococcus aureus</i>	10 / 3
<i>Micrococcus</i> spp.	6,7 / 2
<i>Streptococcus</i> spp.	3,3 / 1
Total	100 / 30

CNS = Coagulase-negative *Staphylococci*

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