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Molecular screening of ovine mastitis in different breeds

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ABSTRACT

Clinical and subclinical mastitis directly affect mammary gland function and have a great economic impact on the sheep and goat dairy industries. The present study explores molecular diagnosis of ovine subclinical mastitis as a faster and more precise screening method compared with microbiology and biochemical techniques to assess the molecular and chemical properties of raw milk samples from healthy animals from 3 breeds of sheep raised in Portugal. Based on 16S ribosomal RNA screening by PCR, milk samples from all sheep were categorized as contaminated (n = 123) or noncontaminated (n = 104). For contaminated milk, different specific primers were used for pathogen identification (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*). *Streptococcus agalactiae* was identified as the most frequent agent. We further assessed whether contaminated versus noncontaminated samples were chemically different in terms of fat, protein, lactose, pH, and solids-not-fat. This molecular screening method allowed rapid and efficient identification of contaminated raw sheep milk, including pathogen identification, before significant alterations in milk chemical properties could be detected. This methodology may lead to a specific and efficient animal treatment and consequently less expensive flock management.

Key words: subclinical mastitis, sheep milk, DNA extraction, PCR-based screening method

INTRODUCTION

In southeastern Portugal, raw sheep milk from different local and exotic breeds is traditionally transformed into cheese. Serpa, a Protected Denomination of Origin cheese by European Union regulations, is a traditional Portuguese cheese with a unique strong aroma and spicy flavor (Alvarenga et al., 2008). The maintenance of milk quality depends greatly on flock health; several livestock diseases may alter milk properties, thus affecting final cheese quality. One of the most common dairy sheep health problems is mastitis—infection and inflammation of the udder that directly affects mammary gland function (Meiri-Bendek et al., 2002; Al-Majali and Jawabreh, 2003). As raw sheep milk is solely used for cheese production, late detection of mastitis may have a negative effect, not only on productivity, but also on final cheese quality, and thus having a negative economic effect. Furthermore, early identification of contaminants in dairy herds might help in formulating efficient strategies to reduce mastitis prevalence. Therefore, assessment and rapid diagnosis of ovine mastitis, with precise pathogen identification, is of great importance.

Mastitis may result from trauma, udder injury, chemical irritation, or bacterial infection, and it is classified into 2 major types: clinical and subclinical (Al-Majali and Jawabreh, 2003; Cremonesi et al., 2006). Clinical mastitis is characterized by presentation of visible signs, and is generally associated with alterations in milk composition, such as increased pH and milk SCC and decreased lactose and total fat contents, leading to a reduction in milk yield and quality (Ogola et al., 2007; Raynal-Ljutovac et al., 2007). Subclinical cases, characterized by the absence of visible signs, are more persistent and highly contagious to the rest of the flock, because animals are asymptomatic (which can lead to late or no diagnosis) while in contact with healthy animals (Al-Majali and Jawabreh, 2003; Viguier et al., 2009). According to Bergonier et al. (2003), the incidence of sheep clinical mastitis is generally less than 5%, whereas that of subclinical cases ranges from 16 to 35%; thus, the overall disease burden is likely to be underestimated, with a huge economic impact on flock management (Leitner et al., 2004) and animal health.

A wide range of infectious microorganisms is known to cause mastitis, generally being classified by reser-

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voir and mode of transmission. Contagious pathogens (the major agents causing subclinical mastitis) such as *Staphylococcus aureus* and *Streptococcus agalactiae* are transmitted among animals by direct contact with infected milk; for example, on animal beds or during the milking process (Al-Majali and Jawabreh, 2003; Mørk et al., 2007). Environmental pathogens such as *Streptococcus uberis* and *Streptococcus dysgalactiae* are generally opportunistic invaders of the mammary gland (Riffon et al., 2001; Cremonesi et al., 2006; Mørk et al., 2007), although *Strep. dysgalactiae* may also present contagious characteristics, spreading from animal to animal (Bradley, 2002).

Different diagnostic methods have been developed to assess contamination status as well as detection and identification of mammary gland pathogens (Riffon et al., 2001; Meiri-Bendek et al., 2002; Cremonesi et al., 2006). According to the National Mastitis Council (1998) standards, currently available microbiology and biochemical techniques for screening and identification of raw milk pathogens requires at least 48 h until the confirmed results. In addition, many of the commercially available microbiological identification systems (such as API20, bioMérieux Inc., Hazelwood, MO) cannot identify, in ovine milk, many of the most relevant mastitis pathogens, such as *Strep. agalactiae*, *Strep. dysgalactiae*, and *Strep. uberis* (Riffon et al., 2001; Viguier et al., 2009).

Because of the limitations of diagnostic tools based on microbiology, the development of molecular biology techniques provides a promising option for rapid animal screening, allowing discrimination between closely related contamination organisms. Raw bovine and ovine milk samples could serve as templates for amplification of specific DNA sequences using PCR (Lipkin et al., 1993; Berri et al., 2000). Moreover, species-specific DNA sequences such as the intergenic conserved ribosomal (**r**)RNA genes (16S or 23S rRNA), or even the entire intergenic spacer region (16S–23S rRNA) of the ribosomal RNA operon, can be used for a rapid (hours) identification of contaminated samples, rather than days, as in microbiology methods (Riffon et al., 2001; Meiri-Bendek et al., 2002; Cremonesi et al., 2006). Several studies on bovine mastitis screening have demonstrated the advantages of molecular biology-based methods compared with traditional microbiology techniques (Riffon et al., 2001; Cremonesi et al., 2006): less time consumed and the ability to identify a wider range of contamination agents were highlighted. Studies describing application of molecular tools for ovine mastitis diagnosis are scarce (López-Calleja et al., 2004; Mafra et al., 2004). Moreover, the studies undertaken did not standardize an ovine raw milk DNA extraction protocol, a significant drawback in the context of routine molecular biology-based procedures. Molecular tools designed for bovine mastitis diagnosis are considerably more developed, with optimized DNA extraction and pathogen identification by PCR-based methods (Riffon et al., 2001; Cremonesi et al., 2006). Currently, efficient and rapid bovine mastitis screening tests have been developed that allow the combination of certain milk properties with molecular biology assays (Riffon et al., 2001).

The present work addresses the molecular diagnosis of ovine subclinical mastitis, assessing the molecular and chemical properties of raw milk samples from different sheep breeds. To the best of our knowledge, this is the first report on screening for ovine subclinical mastitis diagnosis using DNA extraction and PCR-based methods, where 4 different pathogens are tested (*Staph. aureus*, *Strep. agalactiae*, *Strep. dysgalactiae*, and *Strep. uberis*).

MATERIALS AND METHODS

Animals and Samples

The animals used in this study were of 3 different breeds raised in southern Portugal. Merino and Campaniça are local breeds raised under extensive conditions. Today, they are mainly used for meat production, but in the past, milk from these breeds was transformed into high-quality cheese. Today, the Lacaune, of French origin, is the main dairy sheep breed used in the region for cheese production; Lacaune sheep are raised in semi-intensive conditions.

In total, 227 milk samples from the 3 sheep breeds were randomly collected (Table 1) from different flocks in the Baixo Alentejo region of southeastern Portugal. For the Lacaune breed, milk samples were collected in 3 stages of lactation: early, mid, and late lactation. All Campaniça and Merino milk samples were collected in late lactation.

For the purpose of this study, milk samples were collected manually from ewes without visible signs of mastitis just before the afternoon milking. The first streams of each individual milk collection were discarded, and then 25 mL from each half udder (a total of 50 mL) was pooled in the same sterile tube and transported to the laboratory in isothermal boxes (4°C).

DNA Extraction

A raw ovine milk sample (control) was used to test 6 different DNA extraction protocols, protocols I to VI. Protocols I (Cremonesi et al., 2006), II (López-Calleja et al., 2004), III (Murphy et al., 2002), and V (Miller et al., 1988) were performed according to the published