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Identifying the major bacteria causing intramammary infections in individual milk samples of sheep and goats using traditional bacteria culturing and real-time polymerase chain reaction

M. Rovai,* G. Caja,* A. A. K. Salama,*† A. Jubert,‡ B. Lázaro,§ M. Lázaro,‡ and G. Leitner#[1]
*Group of Ruminant Research (G2R), Department of Animal and Food Sciences, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
†Sheep and Goat Research Department, Animal Production Research Institute, Dokki 12311, Giza, Egypt
‡Laboratori Interprofessional Lleter de Catalunya (ALLIC), 08348 Cabrils, Spain
§Vacunek, Ibaizabal Bidea 800, Parque Científico y Tecnológico de Bizkaia, 48160 Derio Bizkaia, Spain
#National Mastitis Reference Center, Kimron Veterinary Institute, PO Box 12, Bet Dagan 50250, Israel

ABSTRACT

Use of DNA-based methods, such as real-time PCR, has increased the sensitivity and shortened the time for bacterial identification, compared with traditional bacteriology; however, results should be interpreted carefully because a positive PCR result does not necessarily mean that an infection exists. One hundred eight lactating dairy ewes (56 Manchega and 52 Lacaune) and 24 Murciano-Granadina dairy goats were used for identifying the main bacteria causing intramammary infections (IMI) using traditional bacterial culturing and real-time PCR and their effects on milk performance. Udder-half milk samples were taken for bacterial culturing and somatic cell count (SCC) 3 times throughout lactation. Intramammary infections were assessed based on bacteria isolated in ≥2 samplings accompanied by increased SCC. Prevalence of subclinical IMI was 42.9% in Manchega and 50.0% in Lacaune ewes and 41.7% in goats, with the estimated milk yield loss being 13.1, 17.9, and 18.0%, respectively. According to bacteriology results, 87% of the identified single bacteria species (with more than 3 colonies/plate) or culture-negative growth were identical throughout samplings, which agreed 98.9% with the PCR results. Nevertheless, the study emphasized that 1 sampling may not be sufficient to determine IMI and, therefore, other inflammatory responses such as increased SCC should be monitored to identify true infections. Moreover, when PCR methodology is used, aseptic and precise milk sampling procedures are key for avoiding false-positive amplifications. In conclusion, both PCR and bacterial culture methods proved to have similar accuracy for identifying infective bacteria in sheep and goats. The final choice will depend on their response time and cost analysis, according to the requirements and farm management strategy.

Key words: mastitis prevalence, small ruminant, subclinical mastitis, real-time polymerase chain reaction

INTRODUCTION

Milk provides the major source of income in dairy farms. Therefore, every disturbance in producing optimal milk quantity and milk quality will reduce farm profitability. Milk quantity and quality are related to genetics (i.e., breed) and environment (i.e., nutrition and management) as well as to animal health (i.e., udder health). Small ruminant production systems vary widely, from traditional hand milking to the most modern computerized milking parlors, and with different dairy breeds, herd sizes, and levels of milk yield. Despite these differences, all sheep and most goat milk is destined for manufacturing dairy products. Therefore, milk quality is key for high-quality dairy products and milk from intramammary infected glands alters its manufacturing ability (e.g., rennet coagulation and curd firmness) for dairy products.

Intramammary infection is one of the main causes of milk production losses (Gonzalo et al., 2002; Leitner et al., 2007, 2008) along with changes in its composition (Leitner et al., 2004a,b). In addition, IMI influences milk coagulation properties, depending on the species of bacteria (Leitner et al., 2006). Of the bacteria involved, some cause clinical infection [i.e., *Escherichia coli*, *Staphylococcus aureus*, and streptococci, whereas the majority cause only subclinical infection with no visible signs (i.e., *Staphylococcus* spp., including *Staph. aureus* and CNS)]. Consequently, to identify infected animals as well as the infecting bacteria, milk sampling and laboratory diagnoses are needed. However, these methods are expensive and the time of sampling is crucial. Conventional bacterial culturing often requires 48 to 72 h for incubation and additional confirmation tests that take time to be completed (Oliver et al., 2004).

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[1]Corresponding author: leitnerg@moag.gov.il

On the other hand, PCR assay kits available on the market for mastitis testing do not require a culture step and can be performed in 3 to 4 h total (Koskinen et al., 2009). Nevertheless, current prices are up to approximately $30/sample. In clinical mastitis, such rapid results should shorten the total duration of treatment, improve the therapeutic outcome, and decrease unnecessary use of antimicrobials (Pyörälä, 2002; Barkema et al., 2006; van den Borne et al., 2010). For clinical cases of mastitis, early detection is critical to help animals to heal and to apply veterinary treatment if necessary. On the other hand, with regard to subclinical infection, the time of laboratory diagnostic procedure is not crucial for chronic forms; however, justification of sampling and testing are based on cost-benefit analysis aiming to improve milk yield and composition.

Bacterial identification by inoculating milk on agar plates is the gold standard of classical bacteriology (Oliver et al., 2004). In the last 20 yr, although DNA-based methods such as real-time PCR and other methods on the market have increased the sensitivity and shortened the time needed for bacterial identification, costs have remained relatively high. Moreover, PCR could also enable the differentiation between various genes; for instance, β-lactam positive and negative. Interpretation of the PCR results is questionable because they may either contain more than 1 bacterial species or give false-positive results for animals free of inflammation (Koskinen et al., 2010). Possible causes of PCR false-positive results could be little bacterial shedding (i.e., less than 3 cfu) or no growth of microorganisms in the culture media or in the contaminated milk (i.e., bacteria coming from other sources such as ovine and caprine). Hence, positive results in a sample showing more than a single bacterial specie from known clinically and subclinically infected glands, or from glands with no inflammation (no increase in SCC) or altered cell distribution, may be either true positive or derived from extramammary contamination of bacteria inhabiting the udder skin and teat canal (Taponen et al., 2009).

The objectives of the current study were to (1) compare the identification of the main bacteria causing clinical and subclinical IMI in individual milk samples taken from sheep and goat udder halves, using traditional bacterial culturing (**BAC**) and real-time PCR and (2) to study the influence of IMI on milk yield and SCC of dairy sheep and goats.

MATERIALS AND METHODS

Animals

The study was conducted at the Experimental Farm of the Servei de Granges i Camps Experimentals (SGCE) of the Universitat Autònoma de Barcelona (**UAB**, Bellaterra, Spain). The Ethical Committee on Animal and Human Experimentation (CEEAH) of the UAB approved all the experimental and animal care procedures.

A total of 108 lactating dairy sheep (parity = 2.9 ± 0.2) of 2 breeds [Manchega (**MN**): n = 56; Lacaune (**LC**): n = 52] and 24 Murciano-Granadina dairy goats (parity = 4.0 ± 1.0) were studied after the weaning of the lambs and after parturition, respectively. Animals were kept under a semi-confinement system, allowed to graze for 6 h daily in an annual Italian ryegrass pasture, and supplemented indoors with alfalfa hay ad libitum (1.27 Mcal of NE_L/kg and 20.1% CP; DM basis) and concentrate at a flat rate of 0.8 kg/d (1.75 Mcal of NE_L/kg and 16.5% CP; DM basis) distributed during milking. Water and a commercial block of vitamins and minerals (Multi-Block; Agraria Comarcal del Vallès, Les Franqueses, Barcelona, Spain) were permanently available in the shelter.

Ewes were machine milked twice daily (0800 and 1700 h) in a double 12-stall parallel low-line milk pipeline milking parlor (WestfaliaSurge Ibérica SL, Granollers, Spain) equipped with recording jars and electronic pulsators at a vacuum of 42 kPa, 120 pulses/min, and 50% pulsation ratio. Goats were milked once per day (0900 h) in the same milking parlor using different electronic pulsators (90 pulses/min and 60% pulsation ratio). The milking routine included machine milking, without udder preparation, but with postmilking teat dipping in an iodine solution (P3-ioshield; Ecolab Hispano-Portuguesa SA, Barcelona, Spain) after cluster removal. Milk yield of individual ewes was recorded weekly (morning and evening) from the weaning of the lambs (35 DIM) to 120 and 135 DIM for MN and LC, respectively. For goats, individual milk yield was recorded weekly from 7 to 280 DIM.

Milk Sampling

Milk samples were taken aseptically by hand milking during the morning milking and submitted to the laboratory within 2 h. Before sampling, teats were disinfected by dipping in an iodine solution (P3-ioshield) and dried with disposable paper towels. The first 3 milk squirts were discarded. Following cleaning, the teats were disinfected with 70% ethanol and left for approximately 1 min to evaporate any remaining alcohol. Milk of the first squirts were again discarded and 3- to 4-mL samples were collected from each udder half into sterile tubes (Eurotubo Deltalab, code 429946; Deltalab SL, Rubí, Spain) for bacteriological testing done on the same day and California mastitis test (**CMT**) examination in situ (Drofilsa; Fatro Ibérica SL, Sant