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Extensive countrywide field investigation of subclinical mastitis in sheep in Greece

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ABSTRACT

The objectives of this work were (1) to investigate prevalence of subclinical mastitis, (2) to identify etiological agents involved, and (3) to study factors potentially predisposing ewes to subclinical mastitis. Milk samples were collected from 2,198 ewes in 111 farms with a total population of 35,925 ewes, in all 13 administrative regions of Greece, for bacteriological and cytological examination. Prevalence of subclinical mastitis was 0.260. Main etiological agents were staphylococci (*Staphylococcus aureus* and coagulase-negative species), which accounted for 0.699 of all isolates recovered; prevalence of staphylococcal mastitis was 0.191. In a multivariable mixed-effects analysis, the primary factor found to be associated with increased prevalence of subclinical mastitis was the management system practiced in flocks (flocks under a semi-intensive system had the highest prevalence). Other factors that were included in the multivariable model were the stage of lactation period (ewes in the 2nd month postpartum showed the highest prevalence) and application of postmilking teat dipping. In contrast, measures taken at the end of a lactation period (e.g., intramammary administration of antimicrobial agents) were not found to have an effect on prevalence of subclinical mastitis. The results confirmed the significance of subclinical mastitis as a frequent problem of ewes, with staphylococci as the primary etiological agent. The findings confirm the multifactorial nature of subclinical mastitis and indicate that its control should rely on many approaches.
Key words: dairy sheep, mastitis, prevalence, risk factor

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INTRODUCTION

In sheep, mastitis is a multifactorial problem, with many bacteria identified as causal agents and many factors accounting for potential predisposition (Gelasakis et al., 2015; Fthenakis et al., 2017). Mastitis adversely affects production and causes financial problems, especially in dairy farms; it has also been recognized as the most important cause of welfare concerns in ewes (European Food Safety Authority, 2014).

In a recent literature review (Gelasakis et al., 2015), it was reported that field investigations on mastitis in ewes were limited in terms of number of animals sampled and number and geographical extent of farms. In the 15 papers reviewed therein, which described investigations in 10 countries, the median number of animals per study sampled was 380, number of farms was 11, and number of milk samples examined was 703 (Gelasakis et al., 2015).

This paper presents results of an extensive, countrywide study on subclinical mastitis in ewes across Greece. The investigation included 111 farms located in all 13 administrative regions of Greece; the total ewe population in these flocks was approximately 35,000 animals. In Greece, sheep production is the predominant form of agriculture, with over 95% of ewes farmed for dairy production. The objectives of this work were (1) to investigate prevalence of subclinical mastitis, (2) to identify etiological agents involved, and (3) to study factors potentially predisposing ewes to subclinical mastitis.

MATERIALS AND METHODS

Sheep Farms

In total, 111 sheep farms in the 13 administrative regions of Greece were included into the study and visited

for collection of samples and information. Veterinarians active in small ruminant health management around Greece were contacted by telephone and asked if they wished to collaborate in the investigation. In total, 25 veterinarians were contacted; of these, 23 (0.92) agreed to collaborate. Farms were selected by the collaborating veterinarians on a convenience basis (willingness of farmers to accept a visit by University personnel for sample collection). The principal investigators (NGCV and GCF), accompanied by an assisting investigator (KSI, DAG, DCO, or APP), visited all farms for sample collection. The location of farms around the country is shown in Figure 1.

At start of each visit, breed of animals in the farm was recorded and an interview was carried out with the farmer to obtain various information regarding udder health management (Appendix Table A1). Further, the veterinarian was asked whether ancillary tests for diagnosis of mastitis were performed in samples from animals in the farm (possible answers: yes/no).

Animal Sampling

In each farm, 20 clinically healthy ewes (secundiparous or older) were selected for sampling. For selection of animals, farmers had been asked to remove primiparous ewes and ewes with known udder abnormalities from the main flock. The remaining animals were walked to the milking area and 20 ewes were selected by using an electronic random number generator (www.randomresult.com) among the first 50 (farms with >100 ewes, n = 99) or 30 (farms with ≤100 ewes, n = 12) animals that walked therein.

A standardized clinical examination of the udder (observation, palpation, comparison between glands) was performed, always by the principal investigator (NGCV; Fthenakis, 1994; Mavrogianni et al., 2005) and the first 2 squirts of secretion were drawn on the gloved hand of an assisting investigator and assessed. All investigators involved in sampling procedures wore disposable, nonsterile latex gloves (Alfa Gloves, Karabinis Medical SA, Peania, Greece). The principal investigator, who examined the animals and collected the milk samples, changed gloves after procedures in each animal were completed and before moving to the next one. If udder abnormalities [e.g., abnormal secretion, mammary nodules (i.e., firm space-occupying structures), papilloma-type lesions] were present, the ewe was excluded from sampling. Animals that were found with abnormalities and excluded were not replaced.

The orifice, edge, and lower half of the body of the teat were disinfected by single-use sterile gauzes, onto which povidone iodine 7.5% (Betadine surgical scrub, Mundipharma Medical Company, Basel, Switzerland) had been poured, followed by wiping off by means of a new sterile gauze; different gauzes were used for each teat. Then, 10 to 15 mL of secretion was collected into a sterile container; separate samples were collected from each mammary gland into separate containers. Milk samples were then drawn directly onto a paddle for performing the California mastitis test (**CMT**).

Transportation of samples to the laboratory in Karditsa was always handled by the principal investigators. Samples were stored in portable refrigerators with ice packs and transported by car; for samples collected in islands, airplane (farms in Crete, Lesvos, or Rhodes) or boat (farms in Cephalonia) transportation, with accompanying luggage (but always ice-packed), was also involved.

Microbiological Examination

Laboratory procedures started within 24 h after collection. Milk samples (10 μL) were cultured using Columbia blood agar plates incubated aerobically at 37°C for 48 h. If nothing had grown, media were reincubated for another 24 h. Bacterial identifications were performed by using standard methods (Barrow and Feltham, 1993; Euzeby, 1997).

In total, 115 CNS isolates [91 from cases of subclinical mastitis (0.200 of such isolates) and 24 from cases of mammary carriage (0.145 of such isolates)] recovered in pure culture during the study were selected at random and identified to species level by using the Vitek 2 automated system (BioMerieux, Marcy-l'-Étoile, France; definitions of subclinical mastitis/mammary carriage are detailed in the "Data Management and Analysis" section below). For selection of isolates for speciation among all those recovered, an electronic random number generator was employed.

Cytological Examination

After sample collection, at ewe-side, all samples were tested by use of the CMT. The test was performed as previously described for ewe milk (Fthenakis, 1995); it was always carried out and scored by the same person (i.e., the principal investigator, NGCV). Five degrees of reaction (negative, trace, l, 2, and 3) were described (Schalm et al., 1971). Milk smears were also produced and dried.

Subsequently, the microscopic cell counting method (**Mccm**; IDF reference method; International Dairy Federation, 1984; Contreras et al., 2007; Raynal-Ljutovac et al., 2007) was performed in 894 samples (0.203 of all samples). The milk smears were stained by the Giemsa method for estimation of leukocyte subpopulations; proportion of leukocyte types therein was