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Association of subclinical mastitis prevalence with sheep breeds in Greece

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The objective of the research described in this Research Communication was to describe potential associations of subclinical mastitis with sheep breeds in Greece. A countrywide survey (2198 ewes in 111 farms) was performed. Prevalence of subclinical mastitis was 0·260. Results did not indicate any difference in the prevalence of subclinical mastitis between farms with pure-bred and farms with cross-bred animals, nor any difference in prevalence between farms with Greek pure-bred animals and farms with imported pure-bred animals. Results indicated that prevalence of subclinical mastitis was smaller in farms with Assaf-breed (0·100) and higher in farms with Frisarta-breed (0·625) ($P < 0·02$). Prevalence of mastitis was smaller in farms with Greek traditional indigenous breeds (0·221) ($P = 0·007$). In a model that included sheep breed and management system in farm, breed emerged as a significant factor for prevalence of subclinical mastitis ($P = 0·003$).

Keywords: Breed, management system, prevalence, subclinical mastitis, sheep.

The predominant type of sheep production in Greece is dairy, with various breeds present around the country. There has been no systematic study of potential association between sheep breeds in the country and mastitis. The genetic background of sheep susceptibility to mastitis has been presented and the role of sheep breeds, as carriers of relevant genes, has been mentioned (Bishop, 2015). There is little work worldwide in relation to the potential susceptibility of sheep breeds to mastitis although Larsgard & Vaabenoe (1993) have indicated some differences between Norwegian sheep breeds, whilst Burriel (1997) has reported that mule ewes were more susceptible to mastitis than Welsh-Mountain ewes (a traditional indigenous British breed). The objective of the work was to describe potential associations of subclinical mastitis with breeds of sheep in Greece.

Materials and methods

In total, 111 sheep farms in the 13 administrative regions of Greece were included in the study and all were visited for collection of samples and information. In each farm, 20 clinically healthy ewes were selected and sampled by use of standardised methods. Bacteriological and cytological examinations were performed in milk samples. Ewes were considered to have subclinical mastitis when a bacteriologically positive milk sample ((a) >10 colonies of the same organism and (b) no more than two different types of colonies) with concurrently increased CMT score (≥'l') plus neutrophil and lymphocyte proportion (≥65% of all leucocytes) was detected.

Mixed-effects logistic regression was employed, using the different farms as 'random effect'. Analysis of variance was employed for performing comparisons between farms in relation to prevalence of subclinical mastitis. Farms with Cephalonia, Crete, Karagouniko, Karystos, Lesvos and Vlahiko breeds were clustered as 'Greek traditional indigenous breeds' and comparisons were repeated. A multivariable model was created using mixed-effects logistic regression with farm as the random effect, which included as variables the management system and the sheep breed.

Detailed description of procedures and techniques employed are in online Supplementary File, item 1. Location of farms around the country is shown in Supplementary File, item 2.

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Results

In total, 2220 ewes were examined and 2,198 were sampled. Among these, 572 were detected with subclinical mastitis; prevalence was 0·260 (95% C.I 0·242–0·279). Prevalence within farm varied from 0·000 to 0·850 (median: 0·250). The most frequently isolated bacteria from ewes with subclinical mastitis were *Staphylococcus* spp. ($n = 531$) (*Staphylococcus aureus* or coagulase-negative species). Less frequently isolated organisms were *Streptococcus* spp., *Corynebacterium* spp., *Escherichia coli*, *Micrococcus* spp., *Mannheimia haemolytica* and *Trueperella pyogenes*.

Of the 111 farms, 58 included pure-bred animals (33 with Greek breeds: Cephalonia $n = 2$, Chios $n = 13$, Crete $n = 4$, Frisarta $n = 2$, Karagouniko $n = 3$, Karystos $n = 1$, Lesvos $n = 5$, Vlahiko $n = 3$, and 25 with imported breeds: Assaf $n = 2$, Lacaune $n = 23$). The other 53 farms included cross-bred animals. In farms with intensive management system, pure-bred animals prevailed (17/26), of which most were imported (11/17). Pure-breeds also prevailed in semi-extensive or extensive management system (16/28), but most were Greek breeds (14/16). In farms with semi-intensive management system, cross-breeds prevailed (32/57). Details of breeds in farms are in online Supplementary File, item 3.

Difference in prevalence of subclinical mastitis between farms with pure-bred and farms with cross-bred animals (0·276 and 0·243, respectively) was not significant ($P = 0·144$). Difference in prevalence of subclinical mastitis between farms with Greek pure-bred animals and farms with imported pure-bred animals (0·284 and 0·265, respectively) was also not significant ($P = 0·240$). The difference in prevalence between farms with imported pure-bred animals and all other farms (0·265 and 0·259, respectively) was also not significant ($P = 0·125$). Similarly, differences in prevalence between farms with Greek pure-bred animals, farms with imported pure-bred animals and farms with cross-bred animals (0·284, 0·265 and 0·243, respectively) were not significant ($P = 0·123$).

When farms with the various pure-breeds were considered, it became evident that prevalence of subclinical mastitis was significantly smaller in farms with Assaf-breed sheep and significantly higher in farms with Frisarta-breed sheep ($P < 0·02$ for both comparisons). Further, there was a significantly smaller prevalence in farms with Karystos-breed sheep ($P = 0·045$) and a numerical tendency for higher prevalence in farms with Chios-breed sheep ($P = 0·125$, not significant). When farms with the six Greek traditional indigenous breeds were clustered together, it emerged that prevalence of subclinical mastitis was significantly smaller in that cluster ($P = 0·007$). All other evaluations did not yield significant differences ($P > 0·250$). Details are in Table 1 and Fig. 1.

Sheep breed emerged from the multivariable mixed-effects model as a significant factor for the prevalence of subclinical mastitis ($P = 0·003$). There was a trend for contribution by the management system ($P = 0·087$, not significant); interactions between breed and management system were not important ($P = 0·845$). Results were similar when calculations were performed after including farms under semi-extensive and extensive management in one cluster ($P = 0·007$, $P = 0·060$, $P = 0·768$, respectively).

Discussion

Lacaune- and Chios-breed animals are popular in Greece. These are sheep with high milk production, thus of importance in the dairy production systems applied in the country, which explains the higher proportion of farms with these breeds. The findings indicate increased penetration of imported breeds, which, in recent years, have been favoured by Greek farmers. Lacaune and Assaf predominate among imported sheep breeds in Greece, as they are animals of increased milk production, higher than indigenous Greek breeds. These animals cannot be adapted to the environment, which is reflected in them being included in farms managed intensively or semi-intensively, where they are sheltered and their needs, especially nutritional requirements, can be controlled and covered. Nevertheless, uncontrolled imports may increase risk for transmission of diseases to the indigenous sheep population; for example, in Spain a large proportion of Assaf animals have been found to be infected with *Small Ruminant Lentivirus* (Minguijon et al. 2015), which may lead to transmission of the pathogen to uninfected flocks in Greece after import. Traditional breeds have also been identified, these being of limited geographical distribution and, mainly, in flocks managed under the semi-extensive or extensive system, which constitute the traditional shepherding forms in the country. These are low-input breeds, with very good adaptability to environmental conditions and able to make excellent use of natural resources and locally produced feedstuffs, which explains their management distribution (Georgoudis et al. 2011). There is evidence regarding genetic relationship between animals of those breeds (Ligda et al. 2009; Georgoudis et al. 2011), thus lending support to clustering these breeds for the statistical analysis.

Bacteriological examination of milk samples is employed for diagnosis of subclinical mastitis, as it is considered to provide precise and exhaustive information on infected mammary glands and pathogen involved. However, it is difficult to implement at a large scale and also has various limitations. Moreover, bacterial shedding is variable and levels may sometimes be too low to be detected by conventional techniques (Rupp & Foucras, 2010). Simple, indirect methods have also been widely applied, based on evaluation of inflammation. The ones most frequently used are somatic cell counting and various indirect tests for their measurement. A difficulty in using somatic cell counting is that factors known to influence somatic cell counting have different magnitude in healthy and infected animals (Detilleux & Leroy, 2000). Further, there is a difference in the types of cells in mammary secretion, which can

Table 1. Importance of breed in prevalence of subclinical mastitis in sheep in Greece

Sheep breeds (no. of farms)	Prevalence	Odds ratio (95% CI)	*P*
(a) Greek breeds considered individually			
Cephalonia (*n* = 2)	0·200	10·723 (0·587–195·918)	0·110
Chios (*n* = 13)	0·318	19·164 (1·145–320·750)	0·040
Crete (*n* = 4)	0·218	11·667 (0·671–202·757)	0·092
Frisarta (*n* = 2)	0·625	67·452 (3·803–1,196·329)	0·004
Karagouniko (*n* = 3)	0·233	12·785 (0·727–224·753)	0·082
Karystos (*n* = 1)	0·000	Reference	
Lesvos (*n* = 5)	0·242	13·305 (0·776–228·228)	0·074
Vlahiko (*n* = 3)	0·267	15·202 (0·869–265·926)	0·062
(b) Greek traditional indigenous breeds clustered together			
Chios (*n* = 13)	0·318	1·640 (1·142–2·355)	0·007
Greek traditional indigenous breeds (*n* = 18)	0·221	Reference	
Frisarta (*n* = 2)	0·625	5·865 (2·950–11·660)	<0·001
(c) Imported breeds considered individually			
Assaf (*n* = 2)	0·100	Reference	
Lacaune (*n* = 23)	0·280	1·554 (0·510–4·736)	0·439

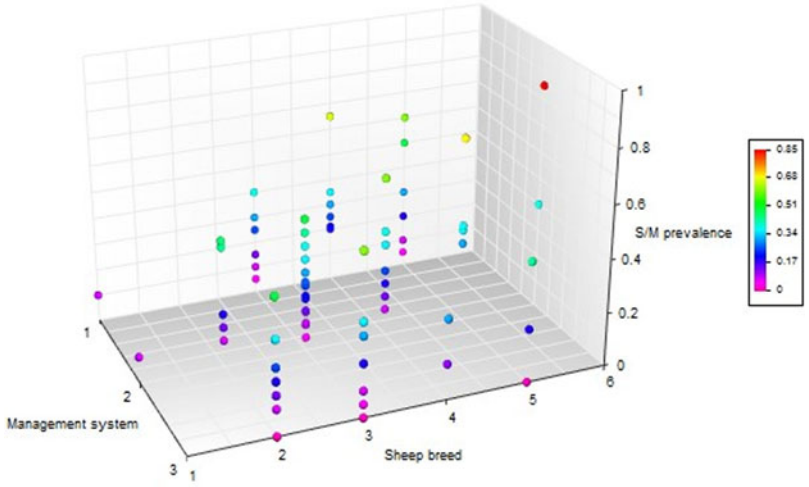

Fig. 1. Scatter plot of results of subclinical mastitis prevalence (*z*-axis) against management system applied in farms (*x* axis) and sheep breed (*y* axis) in 111 sheep farms in Greece. Management system: 1: intensive, 2: semi-intensive, 3: semi-extensive or extensive. Sheep breed. 1: Assaf, 2: Greek traditional indigenous breeds, 3: Cross-breeds, 4: Lacaune, 5: Chios, 6: Frisarta. Subclinical mastitis prevalence: Colour map indicates prevalence of subclinical mastitis.

provide an indication regarding the inflammation. Indeed, the associations between bacteria and somatic cells, particularly of the various types of leucocytes, can be used to better define the disease (Albenzio et al. 2009). The definition of subclinical mastitis used in this study (i.e., combination of a bacteriologically positive milk sample with increased CMT score plus high proportion of neutrophil and lymphocyte) takes that into account and was adopted to overcome shortcomings of the methods described previously.

Present results have indicated increased prevalence of subclinical mastitis in Friesarta-breed farms. Animals of the breed are high-yielding, but, in general, considered to be particularly susceptible to diseases, e.g., respiratory infections. Increased susceptibility to mastitis can be attributed to breed-specific impaired local defence mechanisms in the udder (Fragkou et al. 2007, 2010). Present findings provide field corroboration to the experimental evidence. Traditional Greek sheep breeds have shown reduced frequency of subclinical mastitis. In a broader sense, resistance could be defined as the ability to avoid any infection and/or the quick recovery from an infection (Rupp & Boichard, 2003) and involves different components: avoiding entry of the pathogen into the teat, mounting an immune response capable of limiting its development in the mammary gland and clearing the infection, as well as controlling the pathogenic effects of the infection, such as tissue damage (Rupp & Foucras, 2010). In Karagouniko ewes, lymphoid follicles have been identified in the teat duct and have been repeatedly shown to play a clear protective role against invading

pathogens (Fragkou et al. 2010). Higher allocation of resources to defence mechanisms of ewes afforded by low milk production of these animals can also play a predominant role and contribute to efficient counteraction against invading mammary pathogens. A tendency of increased prevalence of subclinical mastitis in Chios-breed sheep has also emerged. Possible reasons could be the bad udder conformation, which hinders correct milking and contributes to infections (Gelasakis et al. 2012), and the innate peri-parturient immunosuppression associated with macrophage and neutrophil function (Theodorou et al. 2007). Previous studies on other breeds (e.g., Latxa and Sarda) have indeed shown favourable correlations between SCC and udder conformation (Legarra & Ugarte, 2005; Sechi et al. 2007), suggesting that udders with what is perceived to be a good shape would be less affected by subclinical mastitis. In addition, udders with bad conformation can predispose to development of mastitis (Gelasakis et al. 2012). Further, differences in somatic cell counts in milk of healthy animals recorded between sheep breeds (Rupp & Foucras, 2010) can reflect the immunological competence of the respective mammary glands against invading microorganisms and the final result (Albenzio et al. 2012).

In cows, there are many studies detailing genetic resistance to mastitis (discussed by Fragkou et al. 2007). Differences to various defence determinants of susceptible/resistant animals have been reported, e.g. number of blood polymorphonuclear cells after calving, lactoferrin concentration, production of immunoglobulins, production of complement fragment C5a, production and mobilisation of cytokines. There is also information regarding genetic control of lymphocyte mobilisation and role, e.g. heritability (h^2) of T-cell proliferation ranges between $h^2 = 0$ to 0·40, and genetic mechanisms have been identified for production of T-cell and B-cell receptor phenotypes.

Mastitis is a prime target disease to develop breeding for resistance and produce mastitis-resistant sheep (Davies et al. 2009; Bishop, 2015). Genomic selection has been shown to have good accuracy for mastitis resistance in dairy sheep (Duchemin et al. 2012). Our findings have provided evidence of associations of subclinical mastitis with breed, which have only rarely been reported. In Greece, the only breeding programme for genetic control of diseases has been that for scrapie. Certainly, it is more difficult to select for resistance to mastitis, which is a polygenic trait, therefore, selection for a complex of traits is necessary, where many genes with small effects are involved. Given the significance of the sheep industry in the country and the importance of mastitis as a limiting factor in milk production, there is a need to consider genetic improvement for reduced susceptibility to mastitis, as a sustainable means to control the disease.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0022029918000407

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