

Ovine mastitis: Epidemiology, virulence factors and immunorelevant antigens of aetiological micro-organisms

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PhD Thesis, Universidade de Évora, Portugal

Summary

The aim of this work was to investigate the prevalence and aetiology of mastitis in sheep from farms in the region of Évora, Portugal; to detect virulence factors expressed by causative micro-organisms and deepen the knowledge about the defence mechanisms of the mammary gland in sheep. This knowledge may contribute to consider innovative strategies for the prevention and treatment of mastitis, based on immunological methods.

A study was conducted on 18 sheep herds for milk production, whose specific objectives were: [1] Determine the prevalence of clinical and subclinical mastitis; [2] Investigate the aetiology of sheep mastitis; [3] Evaluate antimicrobials susceptibility of isolated micro-organisms. Other items were studied regarding the most frequently isolated etiological agent: [4] assess virulence factors; [5] Identify immunorelevant proteins and investigate local and systemic antibody response in naturally infected sheep.

According to the results, despite the low prevalence of clinical mastitis (CM) which is 1.7%, the prevalence of subclinical mastitis (SCM) is quite high, 32.2%. There was a higher prevalence of mastitis in mechanically milked sheep compared with animals hand milked. The influence of sheep race on the prevalence of SCM has not been confirmed.

Most relevant aetiological agents were coagulase negative *Staphylococci* (CNS), mainly *Staphylococcus epidermidis*. *Staphylococcus aureus* was isolated from cases of SCM, which is a public health concern and *Streptococcus agalactiae* was isolated from cases of CM and SCM, meaning a reduction on milk production in the future.

Isolates of *Staphylococcus aureus* showed greater susceptibility to penicillin than is usually mentioned by researchers. In contrast *Streptococcus agalactiae* isolates revealed less susceptibility to penicillin but increased susceptibility to tetracyclines than usually mentioned. There were more multi-drug resistant isolates of CNS, particularly *S. epidermidis*, than *S. aureus* isolates. In this study three *S. epidermidis* isolates and one *S. aureus* isolate resistant to methicillin were identified.

Concerning the choice of the antibiotic susceptibility test, Kirby Bauer method proved to be reliable for routine *S. aureus* and CNS testing, but the technique should be strictly conducted and caution should be taken with the use of class representatives, specifically not to use clindamycin to decide about lincomycin.

According to our results, the advisable first choice antibiotic is cloxacilin or cloxacilina neomycin association. As to second choice cefuroxime seems to be indicated.

Sheep mastitis isolates of *S. epidermidis* adhere *in vitro* to mammary epithelium cells, but this ability did not influence the inflammatory response in the mammary gland.

The culture in Congo red agar proved to be reliable for the detection of biofilm production expression. Regarding the quantification of the ability to produce biofilm, the microplate method showed some weaknesses to characterize *S. epidermidis* strains.

The expression of the ability to produce biofilm by *S. epidermidis* isolates did not interfere with their *in vitro* adhesion to mammary epithelium, nor influenced the inflammatory response in the mammary gland in response to their infection.

In this study there was no evidence of the production of Staphylococcal enterotoxins, enterotoxin A, B, C and D, nor toxic shock toxin by *S. epidermidis* strains causing mastitis in sheep.

S. epidermidis proteins have been identified that deserve to be evaluated as vaccine antigens, particularly 35-37 kDa proteins.

Systemic humoral response to intramammary infection has not been observed, however local humoral response was evident.

As specific antibodies in sheep milk, IgA is present in both infected and healthy animals, but IgG is only present in infected animals. We consider this information particularly interesting. The confrontation of our results with the facts referred to in other scientific works suggests a hypothesis of explanatory model for the dynamics of immunoglobulins in sheep mammary gland that is described in the thesis.