

## Efficacy of a Novel Vaccine Active against Biofilm Formation by Staphylococci in Protecting Ewes from Staphylococcal Mastitis

*Natalia GC Vasileiou<sup>1</sup>, Katerina S Ioannidi<sup>1</sup>, Vasia S Mavrogianni<sup>1</sup>, Stella N Kalonaki<sup>1</sup>, Teresa Calvo González Valerio<sup>2</sup>, Roger Guix Vallverdu<sup>2</sup>, George C Fthenakis<sup>1</sup>*

<sup>1</sup>University of Thessaly, Veterinary Faculty, Karditsa, Greece

<sup>2</sup>Laboratorios Hipra SA, Girona, Spain

Ovine mastitis is a significant welfare and financial issue in dairy sheep flocks. In milking ewes, the main causative agent of clinical mastitis is *Staphylococcus aureus* and of subclinical mastitis coagulase-negative staphylococci. Biofilm formation is a virulence factor of these bacteria, which contributes to their increased pathogenicity. Objective of the present study was to evaluate under field conditions the efficacy of a novel vaccine, which induces antibodies against the poly-N-acetyl  $\beta$ -1,6 glucosamine exopolysaccharide (PNAG), the main component of the extracellular biofilm matrix of *Staphylococcus*, and acts in preventing slime production and consequently biofilm formation by these organisms. Consequently, vaccination leads to avoidance of biofilm formation by these organisms. The trial was carried out in a dairy sheep farm in Greece. In total, 55 ewes were enrolled in the study, of which 30 were vaccinated (group V) and 25 were unvaccinated controls (group C). The product used is licenced in the European Union (Vimco®) and contains an inactivated slime-producing, biofilm forming *S. aureus* strain. Ewes were vaccinated initially approximately six to five weeks before the expected lambing date, which was followed by a repeat dose three weeks later. Ewes were examined clinically initially during the first 15 days after lambing and then at monthly intervals up to 75th day of the lactation period. At each examination, milk samples were also collected for bacteriological and cytological examination, which were performed by using established techniques. Staphylococcal mastitis was defined as the simultaneous isolation of staphylococci (*S. aureus* or coagulase-negative staphylococci) from a milk sample and concurrently increased cell content in the sample. Incidence risks were calculated for the total period up to the 75th day of the lactation period, as well as for the period 1st to 15th, 16th to 45th and 46th to 75th day after lambing. No cases of clinical mastitis were recorded in any ewe into the study. Further, 3 cases of subclinical staphylococcal mastitis (1 caused by *S. aureus* and 2 by coagulase-negative staphylococcal strains) were identified in group V and 9 cases of subclinical staphylococcal mastitis (1 caused by *S. aureus* and 8 by coagulase-negative staphylococcal strains) in group C. There was clear evidence that, for the period from lambing to the 15th day after lambing, incidence risk (IR) was smaller in group V ewes (IR=0.03) than in group C ewes (IR=0.27), which was statistically significant ( $P=0.007$ ). For the periods 16th to 45th and 46th to 75th day after lambing, incidence risk (IR) was smaller in group V ewes (IR= 0.06 and 0.07, respectively) than in group C ewes (IR=0.15 and 0.22, respectively), although the differences were not significant ( $P>0.18$ ). For the entire trial period, calculated incidence risk was 0.07 for group V ewes and 0.24 for group C ewes ( $P=0.057$ ). There is little documentation regarding the field efficacy of this licenced vaccine against mastitis in dairy ewes. The results confirm the protective effect of the vaccine against staphylococcal mastitis. The effect was stronger during the initial stage of the lactation period, when incidence risk of mastitis is increased, due to the post-partum reduced immunity often observed in ewes.

**Keywords:** Biofilm, coagulase-negative staphylococci, mastitis, sheep, subclinical mastitis, *staphylococcus aureus*