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ABSTRACTS BOOK

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Staphylococcus aureus is the most frequently isolated pathogen from cases of bovine mastitis and can be classified as non-persistent (NP) or persistent (P) according to their ability to adapt to bovine mammary gland. The aim of this study was to evaluate the ability of different *S. aureus* strains (NP and P) to induce cytokine production in MAC-T cells at different post-infection (pi) times. We evaluated the levels of IL-1beta, IL-6 and TNF-alpha production in MAC-T cells infected with *S. aureus* strains NP (3, 17, 48, 179, 806) or P (37, 316, 1595, 5011, 5128). IL-1beta and IL-6 levels were measured by ELISA, while intracellular TNF-alpha production was assessed by flow cytometry. IL-1beta and IL-6 levels, relative to basal cultures and in association to *S. aureus* origin, were analysed using a gamma-linked GLM. All strains were able to induce IL-6 and IL-1beta production (except strain 1595) by MAC-T cells after 2 h pi. Furthermore, an association between cytokine levels and strain origin was observed, where NP strains inducing higher cytokine levels than P strains ($p<0.05$). The ability of *S. aureus* strains to induce TNF-alpha production in MAC-T cells, relative to uninfected cells, was analysed using a T-test. Comparisons between the percentages of TNF-alpha positive cells infected with different strains were made by normalizing to basal production through a linear GML. Three NP strains (3, 17 and 48) and one P strain (1595) were able to induce intracellular TNF-alpha production in MAC-T cells after 24 h pi, where strain 17 inducing the highest TNF-alpha production compared to the other strains ($p<0.05$). In conclusion, although each strain exhibits a unique behaviour upon infection, the origin of the strains influences the activation of MAC-T cells. Overall, an increased level of pro- inflammatory cytokine production by MAC-T cells was observed in response to the NP strains.

607. 228. DISPERSED ASCORBYL PALMITATE (ASC16) AS AN ADDITIVE IN ADJUVANT FORMULATIONS

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Different aspects determine the choice of an adjuvant to generate optimal antibody production while ensuring the safety of the producing animal. In this context, ASC16 that can form safe hydrogels or act as a toxin inhibitor depending on whether it is present at high or low concentrations, respectively. For these reasons, it was proposed to evaluate the effect of dispersed ASC16 (low concentration) as an additive adjuvant in the production of experimental antivenom. BALB/c mice were immunised with Freund's adjuvant in the presence or absence of additive (AFMo and AF) and hydrogel with or without additive (Pa40Mo and Pa40). Afterwards, ELISA, avidity and immunoblotting tests were performed to determine the titre, binding strength and specificity of the antibodies. Finally, we determine the effective dose 50 (ED50) that neutralises PLA2 activity of Crotalus durissus terrificus venom. We used the t-tests for two independent samples to verify if there were significant differences. Our results show that AFMo induced the highest titres (4.07), followed by AF and Pa40Mo (both 3.75), although without significant differences ($p>0.05$), and finally Pa40 (3.27). Notably, the additive ASC16 also improved antibody avidity, with AFMo (4.35 M KSCN) standing out with significant differences compared to the rest ($p\leq0.05$), followed by AF and Pa40Mo (3.21 and 3.17 M KSCN) with no significant differences ($p>0.05$), and finally Pa40 (1.61 M KSCN). In addition, the antibodies recognised the main antigenic components of the venom. The sera produced by formulations with additive presented a better ED50 for PLA2 activity, highlighting Pa40Mo (7.04 µL), followed by AFMo (9.02 µL) with significant differences ($p\leq0.05$) compared to Pa40 (12.20 µL) and AF (12.23 µL). This study shows that formulations with additive ASC16 as an enhancer induce higher antibody titres, improve avidity, have a greater specificity and neutralising effect on PLA2 activity than formulations without the additive.

608. 233. NEW MYCOBACTERIUM BOVIS ANTIGENS FOR USE IN BOVINE TUBERCULOSIS CONTROL STRATEGIES

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Bovine tuberculosis (BTB) poses a threat to livestock production at all levels of production. Mycobacterium bovis is the main causative agent of