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Mastitis was induced in rabbits by *S. aureus* 50 and 06 strains isolated from bovine mastitis cases and study was carried out in two phases. First phase comprised study of pathology of mastitis as infection and second phase consisted vaccination with free cell (FC) and biofilm (BF) vaccines, later challenged by homologus or heterologus *S. aureus*. Both *S. aureus* 50 and 06 strains produced mastitis with clinical symptoms of hyperemia, firmness, bluish discoloration from 24 to 48 hour. The signs were more severe in *S. aureus* 06 than 50. Clinical symptoms in free cell vaccine group were similar nature as in infection trials but absent in biofilm vaccinated groups. The TLC was increased in both infection and challenge trials with marginal increase in heterophils. The Somatic cell count (SCC) was increased in the milk of both *S. aureus* 50 and 06 infected rabbits where as increased SCC was seen in FC group but not in milk of BF groups. Grossly, the mammary gland showed congestion, abscess, and indurations from 24 to 48 hour. Then abscessed cavities were noticed till 144 hours PI, while moderate lesions were noticed in FC group and not in BF group. Histological lesions in infected group consisted of congestion, hemorrhage, heterophil infiltration, desquamation of alveolar epithelial cells, thickening of interalveolar and interlobular septa, and atrophy of alveolus, abscesses, gangrene and massive fibrosis of glands. Histological lesions in FC group were in comparison with infection trials and were absent in BF group. Ultrastructural changes in infection trial showed alveolar epithelial cell damage, loss of nuclear details, and disruption of endoplasmic reticulum, dilated Golgi, vacuolations and thickening of interstitium with electron dense bacteria. These changes were absent in BF group but evident in FC group. Immunohistochemical staining of infected and FC group mammary glands demonstrated bacteria as golden yellow cocci at 24 to 48 hours but absent in BF group. The BF group showed increased number of CD4 T and CD8 T cells on 6, 14 and 21 days post challenge by direct FAT. ELISA titre values were significantly higher in BF vaccinated group compared to FC group.