

Effect of Immunization with InvH Recombinant Protein on Bacterial Shedding, Recovery and Histopathology of *Salmonella enteritidis* Infection

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Background & Objectives: *Salmonella enteritidis*, is the most important pandemic zoonosis in the western world and developed countries. Present vaccines have limited efficacy and hence there is a need to develop stable, effective, and safe vaccines to prevent human and poultry salmonellosis. This organism passes through the intestinal epithelium in membrane-bound vesicles, reaching the lamina propria and cells of the reticuloendothelial system. invH gene, an important part of needle complex in type three secretion system (TTSS) plays important role in efficient bacterial adherence and entry into epithelial cells. The effect of immunization on bacterial attachment and establishment of *Salmonella enteritidis* infection was the purpose of this research.

Methods: In order to study of bacterial shedding, mice were gavaged with 1×10^7 infective live *S. enteritidis* a week following the last immunization. The number of bacteria shed in feces of the immunized and control animals were determined. In recovery of *Salmonella enteritidis* test, Immunized and non-immunized mice were orally inoculated with 10^7 *S. enteritidis*. The animals were sacrificed after 72 hours and spleen, liver and ileum, under aseptic conditions, were removed and homogenized. The samples incubated in 1ml selenite cysteine broth and were subsequently plated on SS Agar plates. In histopathology test, Spleen, liver and ileum removed from mice were fixed in 10% formalin, and embedded in paraffin blocks. Transverse sections ($5 \mu\text{m}$) cut with microtome, were stained with eosin and hematoxylin.

Results: Immunized mice shed high levels of bacteria in outset and reduced gradually to stop completely after nine days. Bacterial loads of $(1.4 \pm 0.33) \times 10^2/\text{gr}$ and $(6.1 \pm 0.41) \times 10^3/\text{gr}$ were detected of spleen and ileum of the positive group respectively while no bacterial load was detected in the same organs of the immunized mice or control groups. Histopathology results showed considerable effect of immunization to inhibit establishment of *Salmonella enteritidis* infection in ileum.

Conclusion: The pathogen has failed to attach the immunized host cells and hence decreased colonization or shedding, also reduced invasion by *Salmonella enteritidis* is noted in this group.

Keywords: Bacterial Shedding; Bacterial Recovery; Histopathology; *Salmonella enteritidis*; InvH