

Title: Cell Invasion, Macrophage Survival and Inflammatory Cytokine Levels Produced among the Closely Related Serovars *Salmonella* Enteritidis and *Salmonella* Dublin

Authors: Campioni, F.¹, Gomes, C.N.¹, Rodrigues, D.P.², Costa, R.G.², Bergamini, A.M.M.³, Tiba-Casas, M.R.⁴, Falcão J.P.¹

Institution: ¹ FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO – USP (AV. DO CAFÉ, S/N – FCFRP/USP, BLOCO S SALA 41 - 14040-903 - RIBEIRÃO PRETO/SP), ² FUNDAÇÃO OSWALDO CRUZ (AVENIDA BRASIL, 4365, PAVILHÃO ROCHA LIMA, 3º ANDAR – MANGUINHOS – 21040-900 – RIO DE JANEIRO/RJ), ³ INSTITUTO ADOLFO LUTZ DE RIBEIRÃO PRETO (RUA MINAS, 877 – 14085-410 - RIBEIRÃO PRETO/SP), ⁴ INSTITUTO ADOLFO LUTZ, SÃO PAULO, SP (AVENIDA DR. ARNALDO, 355, 11º ANDAR, CEP 01246-902, SÃO PAULO – SP, BRAZIL).

Abstract: *Salmonella* Enteritidis and *Salmonella* Dublin are closely related serovars that share antigenic and genetic properties. However, they differ significantly in their pathogenic potential, with *S. Enteritidis* colonizing chickens, and causing gastroenteritis in humans and *S. Dublin* causing systemic infection in cattle and occasionally infecting humans resulting in severe invasive disease. In light of the great importance of the infections caused by these two closely related serovars, the aims of this study were to compare the cell invasion to human epithelial cells (Caco-2) and bovine epithelial cells (MDBK), the capacity to survive and multiply inside human (U937) and chicken (HD11) macrophages of 25 *S. Enteritidis* and 25 *S. Dublin* strains isolated in Brazil between 1983 and 2016. Moreover, the levels of the inflammatory cytokines IL-8, IL-1 β , IL-6, IL-10, IL-12p70 and TNF produced by Caco-2 cells in response to 10 selected strains of each serovar, were analyzed by flow cytometer. *S. Enteritidis* and *S. Dublin* strains studied showed similar percentages of cell invasion either in human epithelial cells (75%) or in bovine epithelial cells (73%). *S. Dublin* strains survived better (80%) in human macrophages than *S. Enteritidis* strains (65%) ($P < 0.05$). Moreover, both serovars had a similar rate of multiplication (3x) inside U937 macrophages after 8 hours of incubation. Regarding the capacity to survive in chicken macrophages, *S. Dublin* had a similar invasion rate (70%) in comparison to *S. Enteritidis* strains (67%) ($P > 0.05$). However, *S. Dublin* strains multiplied better (2x) than serovar *Enteritidis* strains (1x) inside HD11 macrophages after 8 hours of incubation ($P < 0.05$). In addition, 70% of the *S. Dublin* strains analyzed in the cytokine assay did not stimulate the production of any of the cytokines except for IL-8, while 70% of the *S. Enteritidis* strains stimulate the production of all the six cytokines investigated. In conclusion, the better capacity of the *S. Dublin* studied to survive and multiply inside macrophages, as well as the low induction of cytokine production might explain the better ability of strains of this serovar to evade the host immunity response and cause invasive disease in comparison to serovar *Enteritidis* strains.

Keywords *Salmonella* Enteritidis, *Salmonella* Dublin, Cell invasion, Macrophages, Cytokines