

**TITLE:** EXPERIMENTAL DEVELOPMENT OF PROTOCOL FOR ANTIMICROBIAL RESISTANT SALMONELLA ENTERITIDIS STRAIN INOCULATION IN COMMERCIAL EGGS

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## **ABSTRACT**

Several outbreaks of foodborne diseases worldwide are caused by contamination of eggs by *Salmonella*. The re-advent of laying hens in a free-range environment may increase in a significant way the number of contaminated shell eggs, given the greater difficulty in controlling the environmental conditions, thus new indices of related human salmonellosis consumption of eggs and egg products is supposed. Some ways to controlling environmental contaminants may be the natural acids used by egg washing. To evaluate the efficacy of these natural acids the shell must be contaminated in a known concentration, so the aim of this study was to induce a wild *Salmonella* Enteritidis strain resistance to rifampicin, for differentiation by natural contaminants of the product and find the optimal inoculum concentration of this strain to contaminate eggs without shell penetration. The strain of *S. Enteritidis* had its resistance to rifampicin induced based on 20-day protocol described by the *Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas* at Unicamp. Colonies of resistant *Salmonella* obtained were diluted to 10<sup>3</sup> and 10<sup>5</sup> CFU/mL. Therefore, groups of 10 eggs were immersed for 90 seconds in phosphate buffered saline solution (control), solution of *S. Enteritidis* at 10<sup>3</sup> UFC/mL and 10<sup>5</sup> UFC/mL concentration. In addition, the isolation of *Salmonella* in the shell and in the egg internal content was investigated. The induction process to bacterial resistance was efficient. The bacteria infected the shell and the internal contents of the eggs at tested concentrations when incubated at 37°C. The minimum detection limit was 2 CFU/100 µL for the isolation of *Salmonella* in eggshell, so another concentrations and different ways of inoculation must be tested for find the optimal inoculum concentration of this strain to contaminate eggs without shell penetration. Acknowledgments: The authors would like to thank the companies Biosystems and Navelab for the donation of consumer materials used in this research.

**Keywords:** bacterial resistance, cross-contamination, food-borne diseases, salmonellosis