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Differences on the expression of gene *inv*A from *Salmonella heidelberg* isolated from the field and poultry carcasses

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Studies on gene expression have been used in many microorganisms, and quantitative PCR (qPCR) is an important tool. The *inv*A gene is a marker for virulence of *Salmonella* spp. and its expression is related to bacterial invasiveness that could result in severe disease. Thus, the aim of this study was to investigate differences in the expression of this gene in isolates of *S. heidelberg* (SH) of avian origin through the qPCR technique. This study was conducted at the University of the State of Santa Catarina (UDESC), Chapecó city, Southern Brazil. SH samples (n=18) were isolated from Paraná State, Southern Brazil, being 7 from the slaughterhouses (carcasses) and 11 from the field (drag swabs). Bacterial RNA was extracted using the PureLink® RNA Mini Kit (Ambion, Life Technologies, Carlsbad, USA) with subsequent quantification by Qubit® 2.0 Fluorometer (Invitrogen Life Technologies, Carlsbad, USA). The resulting RNA was treated with the enzyme deoxyribonuclease I Amplification Grade (Invitrogen Life Technologies, Carlsbad). cDNA was synthesized using the reverse transcriptase kit with high capacity cDNA (Applied Biosystems, Foster, USA). The cDNA was treated with the enzyme inhibitor RNaseOUT Recombinant Ribonuclease ™ (Invitrogen Life Technologies, Carlsbad, USA). The analysis of *inv*A expression was performed using primers previously described by Rahn et al., 1992. As a reference gene, *16S rRNA* gene was used (Botteldoom *et al*., 2006). For qPCR reactions, the kit SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, USA) was used. Amplification conditions for *inv*A and *16SRNAr* genes were obtained as described by Mustafa and Singh (2013). For both genes, after 40 cycles of amplification, all samples were subjected to analysis of the dissociation curve (melting curve). Each sample was done duplicate with specific to qPCR optical plates in a 96 well thermal cycler in real time CFX96 (Bio-Rad). Amplification results were analyzed using the Bio-Rad CFX Manager software. For gene 16SRNAr a straight line was obtained with a correlation coefficient (R^2) of 0.995 and reaction efficiency (E) of 80.2%. As for the *inv*A gene the value of R^2 was 0.990 and the E value was 83.1 %. All isolates of SH expressed the *inv*A gene. However, the amount of protein expression varied among isolates, where samples from the field had, on average, 2.53 times higher expression compared to those isolated from carcasses. Since *inv*A is related to the capacity of *Salmonella* to cause disease in many hosts, further studies are needed to evaluate the importance of a greater expression of *inv*A and the onset of disease and its degree of severity.

Biography

Lenita Moura Stefani has completed her Veterinary Medicine degree (1993) from the Federal University of Rio Grande do Sul (Brazil), Master's degree in Animal Science (2000) from the University of Delaware/USA, PhD in Veterinary Medicine (2004) from the University of Maryland, College Park, USA, and Post-doctoral (2015) from the University of Maryland, Baltimore Campus, USA. She is a Professor of the Graduate Program in Animal Science at the Universidade do Estado de Santa Catarina (UDESC), Chapecó City, Brazil.

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