



Detection of *Salmonella* Enteritidis in asymptomatic carrier animals: comparison of quantitative real-time PCR and bacteriological culture methods

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ABSTRACT. Quantification of *Salmonella* in asymptomatic carrier animals can be used to assess microbial risk and monitor the level of contamination in domestic animals used for food production. We examined the sensitivity, specificity and accuracy of real-time qPCR, without pre-enrichment or selective enrichment stages, for the quantification of *S. enterica* serovar Enteritidis in resistant mice, as a model of asymptomatic carrier animal. The results were compared with those obtained by traditional bacteriological culture methods, the gold standard test. Two hundred and forty-three samples, including spleen, liver, mesenteric lymph nodes, portions of intestine, intestinal content of the ileocecal portion, and feces, were collected from a group of 27 C57BL/6 mice, that had been intragastrically inoculated with high doses of *S. enterica* serovar Enteritidis. The real-time qPCR assay

presented a consistent linearity of the standard curve ($r^2 = 0.999$), with very low differences between melting temperatures, and low coefficients of variation in intra- (<1%) and interassay (<2%) comparisons. The primers were highly specific; there was no amplification with other *Salmonella* serovars or with DNA from uninfected tissues and feces from mice. The detection limit of the technique was defined as 32 copies of *S. enterica* serovar Enteritidis. A sensitivity of 90%, a specificity of 77% and an accuracy of 79% were obtained. The higher sensitivity of PCR was reflected in a kappa coefficient of 0.41, showing moderate agreement between tests. We conclude that real-time qPCR is a good alternative for diagnostic scanning in asymptomatic carrier animals, due to its high sensitivity and rapidity.

Key words: *Salmonella* Enteritidis detection; Quantitative real-time PCR; Asymptomatic carrier animals; Sensitivity; Specificity; Accuracy