Selection of reference genes in canine uterine tissues

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ABSTRACT. Real-time quantitative polymerase chain reaction (RT-qPCR) is usually employed in gene expression studies in veterinary research, including in studies on canine pyometra. Canine pyometra is a common clinical disease in bitches. When using RT-qPCR, internal standards, such as reference genes, are necessary to investigate relative gene expression by quantitative measurements of mRNA levels. The aim of this study was to evaluate the stability of reference genes and select reference genes suitable for canine pyometra studies. We collected 24 bitch uterine tissue samples, including five healthy and 19 pyometra infected samples. These were used to screen the best reference genes of seven candidate genes (18SrRNA, ACTB, B2M, GAPDH, HPRT, RPL13A, and YWHAZ). The method of KH Sadek and the GeNorm, Normfinder, BestKeeper, and RefFinder software were used to evaluate the stability of gene expression in both pyometra and healthy uterine samples. The results showed that the expression

stability of the candidate gene in pyometra and healthy tissues differed. We showed that YWHAZ was the best reference gene, which could be used as an accurate internal control gene in canine pyometra studies. To further validate this recommendation, the expression profile of a target gene insulin-like growth factor 1 receptor gene (IGF1R) was investigated. We found that the expression of IGF1R was significantly altered when different reference genes were used. All reference genes identified in the present study will enable more accurate normalization of gene expression data in both pyometra infected and healthy uterine tissues.

Key words: Real-time quantitative PCR; Pyometra; Reference genes; IGF1R; Gene expression

