PCR PRIMER DESIGN METHOD FOR DIFFERENTIATING AMONG SALMONELLA SEROGROUPS BASED ON AN ALGORITHM TARGETING GENE-FLANKING REGIONS

Yasukazu Muramatsu¹, Yoshifumi Tsukaya¹, Tetsuya Ikeda², Leo Uchida¹, Yuichi Osa³ and Daiji Endoh⁴

¹Laboratory of Zoonotic Diseases and Laboratory of Veterinary Radiology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu; ²Hokkaido Institute of Public Health, Sapporo; ³Eastern Hokkaido Wildlife Station, Nature Conservation Division, Institute of Environmental Science, Hokkaido Research Organization, Kushiro; ⁴Laboratory of Veterinary Radiology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

Abstract. We applied an algorithm targeting length polymorphisms of intergenic sequences between gene-flanking regions for constructing PCR primer pairs to distinguish among serogroups of *Salmonella*, a major pathogen of humans and animals. From 43 constructed primer pairs, a pair capable in a single-step conventional PCR to categorize five serogroups of *Salmonella enterica* subsp *enterica* into three classes according to amplicon lengths (400, 800, and 900 bp, respectively). Nucleotide sequences of the amplicons were those of flanking regions rfbH and rfbJ. No amplicon was generated in other bacterial genera examined, indicative of the high specificity of this PCR primer pair. As more genetic information becomes available, the smaller number of primer pairs will be required in multiplex-PCR for differentiating Salmonella microorganisms using the novel primer design method.

Keywords: Salmonella, algorithm, gene-flanking region, PCR, serogroup

Correspondence: Dr Daiji Endoh, Laboratory of Veterinary Radiology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu,

Hokkaido 069-8501, Japan. Tel/Fax: +81 11388 4847 E-mail: dendoh@rakuno.ac.jp