TITLE: RAPID DETECTION AND IDENTIFICATION OF YERSINIA ENTEROCOLITICA SEROTYPE O:3 USING A DUPLEX-PCR ASSAY

AUTHORS: RUSAK, L.A. [1,2], REIS, E. M. F[1].; PEREIRA, R.C.L.[1], HOFER, E.[1] , VALLIM, D.C.[1], ASENSI, M.D.[2]

INSTITUTION: [1] LABORATÓRIO DE ZOONOSES BACTERIANAS, INSTITUTO OSWALDO CRUZ, FIOCRUZ (AV. BRASIL, 4365 – MANGUINHOS -PAVILHÃO ROCHA LIMA – 3º ANDAR / SALA 315 - RIO DE JANEIRO, RJ).

[2]LABORATÓRIO DE PESQUISA EM INFECÇÃO HOSPITALAR, INSTITUTO OSWALDO CRUZ, FIOCRUZ (AV. BRASIL, 4365 – MANGUINHOS -PAVILHÃO ROCHA LIMA – 3º ANDAR / SALA 319 - RIO DE JANEIRO, RJ),

ABSTRACT:

Yersinia enterocolitica, a member of the Enterobacteriaceae family, is a zoonotic agent that causes gastrointestinal diseases and some extraintestinal disorders in humans. *Y. enterocolitica* ssp *palearctica* bioserotype 4/O:3 is the primary pathogenic bioserotype in Europe, where it has a high public health relevance. The isolation and identification of *Y. enterocolitica* from various sources on selective media have been seldom successful for several reasons. Trying to avoid the problems associated with traditional culture-based methods, we developed a single duplex PCR assay for the detection and identification of *Y. enterocolitica* ssp *palearctica* bioserotype 4/O:3 using DNA extracted from a source. We combined the primer for *tufA* (elongation factor Tu) with the primer for *rfbC* (the biosynthesis of the O side chain) in one single reaction. A total of 79 *Yersinia* wild trains were selected to evaluate the duplex PCR assay. These strains were composed of 15 *Y. enterocolitica* serotype O:3 strains from human sources, and 64 *Y. enterocolitica* strains of various serotypes from human and animal sources. Nine *Yersinia* type strains were used as positive control for the PCR assay, comprising five *Y. enterocolitica* of several serotypes and four *Yersinia* other species, including *Yersinia. kristensenii*, *Yersinia frederiksenii*, *Yersinia ruckeri*, and *Yersinia pseudotuberculosis*. For negative controls, five strains were used comprising *Escherichia coli*, *Salmonella enterica* ssp e*nterica*, *Citrobacter freundii*, *Enterobacter sakazakii*, and *Proteus vulgaris,* all strains belonged to the *Listeria* collection (CLIST). All the *Yersinia* strains analyzed in this study showed the presence of the *tufA* gene, and the negative controls did not shown cross-reaction with this gene. The amplification of a 405 bp *rfbC* fragment only in the *Y. enterocolitica* serotype O:3 samples showed that this gene is an excellent serotype O:3 marker for diagnostic purposes. The results of multiple alignment of the *tufA* primers with the closest *tufA* sequenced fragments from NCBI database, in and out of the *Yersinia* genus, corroborate with the duplex PCR assay results. This assay could be a suitable screening method for the rapid detection and identification of *Y. enterocolitica* serogroup O:3, other serotypes, and other *Yersinia* species. We anticipate that this assay could be a useful tool for hospital and veterinary surveillance studies on *Yersinia* worldwide.

Keywords: *Yersinia enterocolitica*, detection, Identification, PCR, zoonosis.

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