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Development of a real-time multiplex PCR assay for detection of *Salmonellae* in chicken samples

PHD

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Abstract:

Salmonella is the pathogenic agent of salmonellosis, a major cause of enteric illness and typhoid fever. *Salmonella* Typhimurium infects poultry causing significant losses, serovar 1, 4, [5], 12: i: - is considered a monophasic variant of *S*. Typhimurium that increasingly important as public health risk. A total of 58 *Salmonella* isolates were reidentified by slide agglutination test according to White-Kauffmann- Le Minor scheme. A duplex syber green real time PCR was applied for detection of genus *Salmonella* and *S*. Typhimurium using *16S rRNA* and *fli*C genes. All strains harbor *16S rRNA*. Twenty one strains harbor *fli* C gene that including *S*. Typhimurium (12), *S*. Kentucky (6) , *S*.1,4,[5],12:i:- (1) , *S*. Lagos (1) and *S*. Kedougou (1). A duplex Tagman real time -PCR was performed for differentiation between biphasic *S*. Typhimurium and monophasic variant 1,4,[5],12:i:- using *flj*B1,2 and *fli*B / IS200 in the Fli A. B intergenic region. Ten strains out of 12 *S*. Typhimurium harbor *flj* B1,2 however strain *S* 1,4,[5],12:i:- don't possess this gene. While 13 strains were positive to *fli*B / IS200 (12 *S*. Typhimurium and *S* 1,4,[5],12:i:-). The *fli*B / IS200 is specific gene for *S* .Typhimurium (biphasic and monophasic). These results indicate that two strains serologically confirmed to be *S*. Typhimurium and *S*.1,4,[5],12:i:- don't possess *flj*B1,2 and have *fli*B/ IS200 genes are monophasic variants by a duplex Tagman real time -PCR. It was noticed that prolonged subculture and or repeat phase inversion method leads to formation of flakes that in turn cause wrongly serotyping identification. Real time –PCR is rapid and can be used to identifying and differentiation between biphasic and monophasic *S*.1, 4, [5], 12: i: - *S*. Typhimurium.

Keywords: Biphasic and monophasic *S.* Typhimurium, *fljB1,2* gene, Real - time PCR, *Salmonel*

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