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Developing a New PCR Approach to Distinguish between Rev1 Vaccine Strain and Field Strains of *Brucella melitensis*

Background

Brucellosis that is caused by *Brucella melitensis* is a serious public health problem in Palestine. This zoonotic disease is primarily affecting small ruminants however it can be transmitted to humans causing a serious illness known as Malta fever. *Brucella melitensis* 'Rev1' is an avirulent strain that is widely used as a live vaccine to control Brucellosis in small ruminants. However, Rev1 vaccination can interfere with the diagnosis of *Brucella melitensis*. In addition, shedding of Rev1 in milk after vaccination or after abortion in pregnant animals can increase the risk for human infection. Despite the great need for an accurate assay to differentiate between the vaccinated and infected animals, Rev1 genotyping is still a challenging issue.

Materials and Methods

To identify new discriminatory genetic markers for Rev1 vaccine strain, its full genome sequence was compared with the genome sequences of 36 different *Brucella melitensis* strains that were obtained through various biological databases. A selected group of 12 potential markers were experimentally verified by a novel bi-directional allele-specific PCR for each one. The PCR tests were performed using gDNA of Rev1 strain versus reference strains representing biovar 1, 2 and 3 of *B. melitensis*. The validated Rev1-specific markers were used to analyze a panel of 30 field isolates that were collected from infected animals through the Central Veterinary Laboratory.

Results

The bioinformatic analyses revealed 26 genetic alterations that are exclusively present in Rev1 genome. The identified alterations were remarkably found in different genes and 8 of them are synonymous while 18 are nonsynonymous mutations. Interestingly, only 3 mutations occur within known virulent genes while the pathogenicity of the other affected genes is not characterized yet. The PCR results of the 12 tested alterations confirmed the authenticity of 11 of them as Rev1-specific genetic markers.

Conclusion

We discovered a group of novel genetic markers that are specific for Rev1 vaccine strain. A selected subset of the identified markers, were successfully used to develop a practical and cost effective PCR assay that can differentiate Rev1 vaccine strain from other field strains. The developed assay can be used to examine the stability and safety of the commercial vaccine.