Identification of Novel Genetic Markers that can Distinguish Rev1 Vaccine Strain from Field Strains of *Brucella melitensis*

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Background

Brucellosis 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 human causing a serious illness known as Malta fever. *Brucella melitensis* 'Rev1' is an attenuated strain that is widely used as a live vaccine to control *Brucella melitensis* infection 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 11 potential markers were experimentally verified by sequencing using a commercial Rev1 vaccine 'EV.1' from Laboratorios Ovejero, Spain. We then designed a novel bi-directional allele-specific PCR for each one of the confirmed markers. 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 Palestinian Central Veterinary Laboratory.

Results

The bioinformatics analyses revealed 26 genetic alterations that are exclusively present in Rev1 genome: 8 are synonymous and 18 are nonsynonymous mutations. The mutations were remarkably found in different genes. Interestingly, only 3 mutations occur within known virulent genes while the pathogenicity of the other 23 affected genes is not characterized yet.

Using sequencing technique, 9 out of the 11 potential markers were confirmed as true genetic alterations of Rev1. In addition, the results of the bidirectional PCR show that most of the 9 markers are amenable to develop a practical technique to distinguish between Rev1 and other field strains. Examination of a group of 30 field isolates using the developed bidirectional PCR assays revealed that all the 30 isolates are field strains.

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.