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GI_{FeGSH}: A New Genomic Island Might Explain the Differences in *Brucella* Virulence

Tara Wahab[1,2], Astrid Skarp[3], Viveca Båverud[1,4], Rene Kaden[1,3,4]

[1]Swedish Forum for Biopreparedness Diagnostics (FBD), Umeå, Uppsala and Solna, Sweden
[2]Public Health Agency of Sweden, Solna, Sweden
[3]Department of Medical Sciences, Uppsala University, Uppsala, Sweden
[4]National Veterinary Institute, Uppsala, Sweden

Email: rene.kaden@akademiska.se

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Abstract

An imported dog was confirmed to be positive with canine brucellosis in Sweden in 2010. The whole genome of *Brucella canis* SVA10 was subjected to phage analysis (WGS-PA) and was assigned to the Asian *B. canis* cluster. Further analysis indicated that the genome of *B. canis* SVA10 is smaller compared to genomes of the same species. A 35,781 bp genomic island (GI) was found to be absent in strain SVA10 which was detected by read mapping the paired reads to the genome of *B. canis* ATCC 23,365^T. The lacking genes of genomic island GI_{FeGSH} are mainly coding for iron uptake enzymes and parts of the glutathione pathway. A screening of all available whole genome sequences of *Brucella* strains confirmed that GI_{FeGSH} is also missing in four more strains of *B. canis* but present in several strains of *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. microti*, *B. pinnipedialis*, and *B. ceti.* Parts of the GI were present, but scattered in two other *B. canis* strains. The aim of this study was to find differences in the genomes of *Brucella* which might explain former described differences in virulence. The analysis was extended to all available *Brucella* genomes after the detection of a genomic island in strain SVA10.

Keywords

Zoonoses, Brucellosis, *Brucella canis*, Genomic Island

1. Introduction

The genus *Brucella* currently consists of 11 species of which *B. melitensis*, *B. abortus*, and *B. suis* are further classified in different biovars. The biovar concept is useful especially regarding epidemiological source tracing queries. However, the genetic divergence within the whole genus *Brucella* is very low and makes

genotyping challenging. Phylogenetic analyses based on 16S rDNA commonly used for bacterial speciation are not possible with the *Brucella* species since they share 100% identical 16S rRNA genes. Comparing the whole genomes of all *Brucella* species with DNA-DNA hybridization, the similarity is still between 87% and 100% [1]. Due to the high genetic homology the genomospecies concept was recommended for the genus *Brucella* [2]. However, to avoid confusion not at least in medical diagnostics, The International committee on systematic bacteriology, Subcommittee on the taxonomy of *Brucella* recommended to continue applying the former vernacular names for the nomen species [3]. Those taxonomic issues are showing the difficulties that exist due to the high genetic homology between the *Brucella* species. Consequently, the subdivision of *Brucella* species in distinguishable strains is even more challenging.

Brucella canis infects dogs and humans and is together with *B. melitensis*, *B. abortus*, and *B. suis* one of the more dangerous *Brucella* species regarding zoonotic potential, infectious dose and global distribution. Sweden is officially free of brucellosis and there has only been one case [4] and one outbreak of canine brucellosis [5], both caused by imported dogs. *B. canis* strains can either be assigned to the Africa, America, and Europe AAE group (AAE group) or to the Asian cluster (A group) by whole genome sequencing-phage analysis (WGS-PA) [5]. The WGS-PA cluster assignment is based on the type and number of remaining prophage fragments that are not completely removed during bacteria's evolution. The causative strain of the Swedish brucellosis outbreak in 2013 was assigned by WGS-PA to a cluster of strains that are mainly distributed in the AAE group. *Brucella* strains sometimes contain genomic islands (GI), which range in size from 7 to 44 kilobases (kb) [6]. The GIs and even the same GIs can be present in different *Brucella* species and known differences in pathogenicity and virulence might be explained by genes of the GIs [6] [7]. Each virulence gene can theoretically be located on a GI and due to the fact that many GIs are not stable even strains of the same species might be different regarding the virulence properties. An example for a gene which leads to a higher virulence and a higher efficiency in colonizing the host is the horizontally acquired gene coding for γ-glutamyltranspeptidase [8]. The gene product regulates the glutathione pathway and thus the antioxidant regulation in the host cells.

The aim of this study was a detailed investigation of the genome of the *B. canis* strain SVA10, including screening for genomic variations as well as the comparison of the SVA10 genome to other *Brucella* species genomes regarding potentially genes which might explain differences in *Brucella* virulence.

2. Material and Methods

2.1. *Brucella canis* Strain SVA10

Brucella canis strain SVA10 was isolated at the National Veterinary Institute of Sweden from an American Staffordshire terrier imported from Poland [4]. An aliquot of the original frozen stock was cultivated on Farrell agar [9].

2.2. Whole Genome Analysis

A validated workflow for sequencing and analysis of bacterial samples was chosen for whole genome sequencing (WGS). The DNA of *B. canis* strain SVA10 was extracted from cultivated colonies using an EZ-1 extraction robot and EZ-1 DNA tissue kit (Qiagen, Hilden, Germany). The libraries were prepared with a Nextera XT sample preparation kit (Illumina, San Diego, CA) which allows to proceed samples with a DNA concentration of 0.5 ng/µl. WGS was performed using a 2 × 300 paired-end run on an Illumina MiSeq platform (San Diego, California, USA).

The reads were *de novo* assembled using the Mira plugin version 1.0.1 in Geneious version 8.1.7 [10]. The average nucleotide identity (ANI) as well as the mol% G+C content were determined using the Gegenees software version 2.0 with a score threshold of 20% [11]. Screening and annotation of phage genes was done using PHAST [12]. The annotation of genes as well as the assignment of genes to pathways was done by the Kyoto Encyclopaedia of Genes and Genomes database [13] [14] in addition to the annotation pipeline of the National Center for Biotechnology Information (NCBI). The Mauve version 2.3.1 plugin [15] was used in Geneious for the alignment and positioning of sequence features. The whole genome sequences of all other *B. canis* strains with available WGS data in the NCBI database were downloaded from NCBI (**Table 1**).

Table 1. Summary of all *Brucella canis* strains with available WGS data (2016), origin of the strains, assignment to the WGS-PA cluster and presence of the genomic island GI_{FeGSH}.

B. canis strain	NCBI Accession number	Origin			Presence of GI_{FeGSH}	WGS-PA cluster
		Source	Collected	Geographical		
ATCC 23365T	NC_010103	dog	1968	n.a.	+	AAE
RM6/66T	CP007758	dog	1968	n.a.	+	AAE
SVA13	CP007629	dog	2013	Sweden/Spain	+	AAE
Oliveri	HG803175	dog	n.a.	Columbia	+	AAE
04-2330-1	AXNG01000001-004	n.a.	n.a.	n.a.	+	AAE
96-7258	AXNF01000001-004	n.a.	n.a.	n.a.	+	AAE
CNGB 1324	AQMZ01000001-002	human	2008	Argentinia	+	AAE
CNGB 513	AQJZ01000001-007	human	2001	Chile	+	AAE
CNGB 1172	AQMY01000001-002	human	2006	Columbia	+	AAE
F7/05A	AQNA01000001-003	dog	2005	South Africa	−	AAE
HSK A52141	CP003174	dog	~2011	Korea	+/−	AAE
SCL	LGAQ00000000.1	dog	2008	Chile	+/−	A
UK10/02	AQNB01000001-002	n.a.	2002	n.a.	+	A
79/122	AQJY01000001-005	dog	1979	Japan	−	A
118	AMOZ01000001-154	human	2008	China	−	A
BCB018	ALOJ02000001-170	human	1988	China	−	A
SVA10	MAXW00000000	dog	2010	Sweden/Poland	−	A

+: GI is present; −: GI is absent; +/−: GI is scattered/incomplete, n.a.: no data available.

3. Results and Discussion

The reads were assembled into 12 contigs which were deposited in the NCBI database with the accession numbers MAXW00000000, Bioproject PRJNA328097 and Biosample SAMN05363686.

The range of the genome size of *B. canis* is 3,217,060 bp (strain F7/05A) to 3,318,660 bp (strain Oliveri). The genome of *B. canis* strain SVA10 consists of 3,264,482 bases with a G+C content of 57.24%. The examined strain has one of the smallest genomes of all available sequenced *B. canis* strains. In addition to the *de novo* assembly, the sequence reads of strain SVA10 were mapped against the whole genome sequence of the type strain of the species (ATCC 23,365^{T}; 3,312,769 bases) with the aim to detect the lacking genes of the missing 48,287 bases. The main differences between the two strains regarding lacking genes were observed at chromosome II (**Table 2**). A genomic island, called GI_{FeGSH} was missing in strain SVA10 compared to strain ATCC 23,365^{T} beginning on genome position 625,001. The size of GI_{FeGSH} is 35.8 kb with 36 coding sequences (CDS) and a DNA G+C content of 57.6 mol%. The sizes of the GI and of the annotations in **Table 2** were calculated in Geneious version 8.1.7 [10]. It was confirmed by blasting the predicted tCDS of GI_{FeGSH} against the predicted tCDS of SVA-10 that no alternative amino acid sequences exist in strain SVA10 that would be able to compensate for the proteins encoded on the lacking GI_{FeGSH}.

Two major systems, coding for a Fe^{3+} uptake system and a glutathione pathway were a striking feature on GI_{FeGSH} (**Table 2**). The iron uptake in general is either realised by the water-soluble Fe^{2+} or in case of Fe^{3+} by siderophores which are small molecules that are able to bind the iron outside of the cell. The siderophore can either be actively transported through the cell membrane or it binds to receptors outside of the cell followed by iron reduction to Fe^{2+}. In that way Fe^{3+} becomes available in environments where no dissolved Fe^{2+} is present. However, due to the availability of Fe^{2+} in the host cells there is no need to make Fe^{3+} available, which also means that Fe^{3+} reduction and uptake are not necessary as long as the bacteria are in the host. Generally, redundancy between iron utilization systems is found in different bacteria and thus absence of one system may not impair the organism's ability to colonize or infect its host. However, the lack of these genes becomes relevant in terms of survival outside of the host, which could allow for extended survival times and increase the chance of infection.

The second predominant cluster of genes that are not present in the genome of *B. canis* strain SVA10 are genes that are regulating the glutathione pathway (EC:1.8.1.12, EC:3.4.11.2, EC:6.3.1.9, EC:3.5.2.9). Glutathione is an antioxidant and uptake and utilization systems are present in several bacterial species [16]. Horizontal acquisition of glutathione utilization has been described before in other bacteria; for example, *Campylobacter* strains are able to use glutathione by using the horizontally acquired gene coding for *γ*-glutamyltranspeptidase and such strains are more efficient colonizers of the mouse intestine [8]. The ability to use glutathione could be an advantage in *Brucella*, and may increase colonization and infection potential. Therefore, the presence of the glutathione pathway

Table 2. Genes of the genomic island GI_{FeGSH} which is present in *Brucella canis* strain ATCC 23,365^T and absent in *Brucella canis* strain SVA10.

Gene	Leng
ABC transporter permease	870
Adenine deaminase	1800
Adenine permease	1293
Aldehyde dehydrogenase	1446
Amino acid ABC transporter substrate-binding protein	774
Aminotransferase	>16
Aminotransferase	855
Branched-chain amino acid ABC transporter	717
Branched-chain amino acid ABC transporter permease	1000
Cytidine deaminase	600
Enterobactin ABC transporter permease	954
FAD-binding dehydrogenase	1656
Glycerol-3-phosphate transporter membrane protein CDS; sn-glycerol 3-phosphate ABC transporter permease	849
Glycerol-3-phosphate transporter permease CDS; sn-glycerol 3-phosphate ABC transporter permease	875
Guanine permease	>778
Guanine permease	>449
Hemolysin III	750
Insertase	>11
Iron ABC transporter ATP-binding protein	759
Iron ABC transporter permease	963
Iron ABC transporter substrate-binding protein	970
Leu/Ile/Val-binding protein homolog 6 CDS; amino acid-binding protein	1173
Membrane protein	762
Membrane protein	489
Peptidase	1041
Permease CDS	1772
Ribonuclease P CDS; ribonuclease P protein component	>11
Sn-glycerol-3-phosphate-binding periplasmic protein UgpB	1302
Sugar ABC transporter ATP-binding protein	1056
Sugar ABC transporter permease	>10
Transcriptional regulator	609
Transporter	918

in a few strains might explain why some *B. canis* strains are more virulent than others and why they may differ in zoonotic potential. Additional genes related to bacteria's virulence that were found on GI_{FeGSH} are Hemolysin III and Peptidase.

The necessity of additional genomic content in *B. canis* is disputable since obligate intracellular as well as facultative intracellular pathogens show a trend to reduce their genomes during evolution [17]. Therefore, features such as iron and glutathione utilization may be important during environmental survival or to

exhibit a hypervirulent potential. Future characterization studies of these features can shed light on the importance of the features described here and complement virulo- and epidemiological typing of *B. canis*.

Not much is known about the epidemiology of human infections caused by *B. canis* [18] [19] [20]. Thus, the strains with and without GI_{FeGSH} were clustered and analysed regarding phylogeny and epidemiological parameters. The analysis of all available whole genome sequences of *B. canis* strains showed no evidence for epidemiological links between the strains that are lacking GI_{FeGSH} (**Table 1**).

The detection of GI_{FeGSH} which contains potential virulence genes gives the possibility to assess the effect to the host, the possible transmission as well as the consequences of a *Brucella* infection. Curing brucellosis caused by bacteria containing GI_{FeGSH} is expected to be more challenging and the bacteria might affect the host cells with a higher efficiency.

GI_{FeGSH} might be applicable for source tracing if a strain contains unique scattered parts of GI_{FeGSH} in their genomes as the strains HSK A52141 and SCL (**Table 1**, **Figure 1**).

Due to the high genetic homology it is difficult to find epidemiological markers within the genomes of several Brucella species. A new whole genome sequenced strain of *Brucella canis* (SVA10) which was derived from an infected dog imported from Poland to Sweden was analysed and assigned to the Asian WGS-PA cluster [5]. A genomic island called GI_{FeGSH} was detected in strain ATCC 23,356^T by comparative genomics. GI_{FeGSH} which encodes genes related to the iron uptake and the glutathione pathway was also detected in other *Brucella* species and *B. canis* strains but it was found to be absent in the examined strain SVA10 as well as in a few other *B. canis* strains. GI_{FeGSH} may therefore be useful as an epidemiological marker in *B. canis* if the GI is stable.

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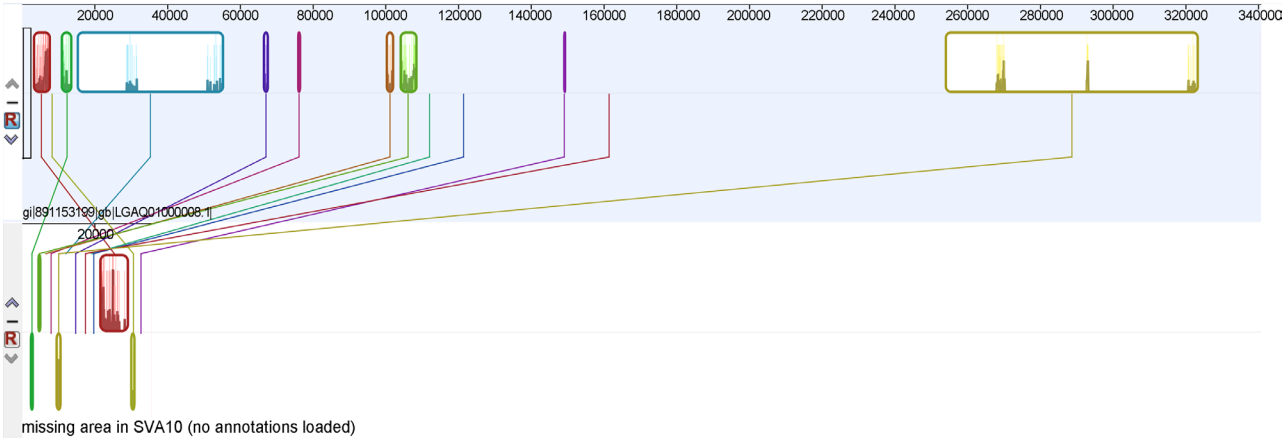

Figure 1. Scattered presence of the genomic island GI_{FeGSH} of *Brucella canis* strain SCL [LGAQ00000000.1]; MAUVE alignment with *Brucella canis* strain ATCC 23,365^T [NC_010103].

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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