Genomic comparison of *Histophilus somni* strains shows genetic drift

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Introduction

Histophilus somni is a gram-negative, non-motile, non-encapsulated, coccobacillus commensal bacterium associated with nasal cavities of cattle. *H. somni* is an opportunistic pathogen that complicates viral infections and increases the severity of infection in association with other bacterial agents. *H. somni* can infect several organs once it becomes blood-borne, and is often associated with *Mannheima haemolytica* and *Pasteurella multocida*. *H. somni* affects the respiratory, genital, nervous (thrombotic meningoencephalitis, TME), circulatory and musculoskeletal systems (joints), either individually or together. Currently there are 22 USDA-licensed *H. somni* vaccines licensed by 5 companies for the US market; however, many of these are no longer being manufactured or marketed. The current manufactured vaccines are heavily used, with 69.7% of all large feedlots in the US vaccinating cattle against *H. somni* (USDA, 2013). Surprisingly, their ability to confer immunity in the production setting is modest or unknown. One of the reasons for perceived low protection is high genetic diversity and genetic-drift among *H. somni* strains. In order to understand the genetic-drift and select candidates for inclusion in autogenous vaccines we performed whole genome sequencing (WGS) and comparative mapping analysis of 30 *H. somni* field isolates (named NPL#) and 2 avirulent mutant strains to the reference strains 129PT commensal isolate and 2336 and HS91 pathogenic isolates.

Materials and Methods

All the *H. somni* isolates were grown in Columbia broth to log phase and the genomic DNA was extracted using a Bacterial Genomic DNA Purification Kit (Edge Biosystems, Gaithersburg, MD), quantified and purity checked on a Nanodrop. Genomic DNA libraries from all strains were prepared using a Nextera XT kit (Illumina). Whole genome sequencing was performed on a MiSeq instrument using 2x150bp chemistry. Once sequenced, to compare 129PT, a commensal phenotype isolate from the database against the 2336 virulent phenotype isolate (NCBI accession # CP000947), pseudo-reads of 129PT were generated using the ART read simulation software (http://www.niehs.nih.gov/research/resources/software/biostatistics/art/). Finally, samtools was used to calculate mapping coverage for each position against the 2336 reference genome. This was used to calculate the percentage of bases that had coverage for each gene. Genes that contained less than 80% of bases were called absent for that respective isolate. Two analyses were conducted on missing genes against 2336.

Results

The overall analysis indicated a genetic-drift in recent *H. somni* isolates when compared to historical isolates. The recent isolates were only 82% identical at whole genome level to the historical isolates (2336 or HS91 another pathogenic phenotype reference strain). This indicates a genetic-drift and possible lack of vaccine protection by older isolates in currently available commercial vaccines. Among the isolates, 129PT lacked many virulence and virulence-associated genes in comparison to the 2336 virulent phenotype. The more recently isolated *H. somni* strains had fewer genes than the older isolates or 2336. When comparing the genes that are absent in the first and second analyses relative to 2336, 129PT the commensal, lacked most of those genes associated with pathogenicity and/or virulence than any other isolate.

Significance

The data indicates that a combination of a number of genes may be required to make an isolate an effective vaccine or challenge candidate, and the loss of only a few genes can attenuate the strain enough to be avirulent. The *H. somni* population does seem to show genetic-drift over time and the industry needs to evolve in order to maintain relevant vaccines to match field strains. The data strongly supports the importance of autogenous vaccines, as well as, periodic re-evaluation of commercial vaccine efficacy.