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August 30, 2020

Progress Report

Project: Detection and analysis of *Streptococcus zooepidemicus*, a recently emergent pathogen of Pigs in PA

Objective: In this proposal we aim to optimize a PCR procedure for identification of Strep zoo in swine, including determining appropriate samples for reliable detection of Strep zoo. Due to the strong possibility of genetic mutation driving higher virulence of Strep zoo, we also propose to characterize the Strep zoo strains in PA by whole genome sequencing

Results and Summary

Two *Streptococcus zooepidemicus* were isolated in December 2019 at Pennsylvania from a swine herd, which experienced high mortality. The pure cultures were confirmed as *S. zooepidemicus* using the conventional biochemical tests and whole genome sequencing. The raw reads have been submitted to the SRA database under the BioProject accession number PRJNA591128. The annotated full genomes of the *isolates* have been deposited in GenBank under the accession numbers JABDID000000000 and JABMIH000000000.

Based on comparative genome analyses we identified a gene *SzM* was conserved among only in the virulent strains of *S. zooepidemicus* . Primers and probe targeting 85 bp region of the *SzM* gene were designed using Primer Express v. 3.0.1® (Applied Biosystems). We designed a novel qPCr assay comprising forward primer (5' – AAGTCGTTGCTCAACTTCATCTATTAAC – 3'), reverse primer (5' – TAGGTAATGACCGTCCTAATGATGTT – 3') and the probe (5' – 6FAM-AGTTTAACCCTCTTGATCTAT-MGBNFQ – 3'). The optimal cycling conditions were standardized as: 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 45 s, with data collection at the end of each 60°C step. Additionally, NCBI Primer-Blast® analysis was used to confirm the specificity of primers by confirming the absence of targets other than virulent strains of *S. zooepidemicus* in the nucleotide sequence database.

A probe based real-time PCR assay developed targeting the highly conserved virulence factor, *SzM of S. zooepidemicus* provides a highly specific means to make a rule in diagnosis of the virulent S. *zooepidemicus* infection. The analytical sensitivity corresponding to the lowest limit of detection was determined as 20 fg of the target DNA. For the first time, we developed this probe-based PCR assay which can differentiate the virulent *S. zooepidemicus* isolates from both avirulent *S. zooepidemicus* and *S. equi* isolates. This assay also forms an important tool in the quest for animal species which could act as reservoirs for *S. zooepidemicus*.

In summary, this novel assay which can give the result in less than 4 hours provides a practical solution to the hitherto unsolved problem of diagnosing virulent *S. zooepidemicus.* The results are currently being written up for a peer reviewed publication.

Sincerely,

Suresh Kuchipudi